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PHYSIOLOGICAL REVIEWS

VOL. VIII OCTOBER, 1928 No. 4

A REVIEW OF HYPOTHESES OF THE STRUCTURE OF PROTEINS[1]

HUBERT BRADFORD VICKERY AND THOMAS BURR OSBORNE

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Recent investigations of proteins and their decomposition products have given rise to considerable speculation upon the constitution and structure of the protein molecule. The great interest that is now being taken in the chemistry of proteins and the apparent widespread acceptance of some of these newer conceptions have made it advisable to enquire somewhat closely into the evidence upon which they rest. In order better to appreciate the background from which the newer ideas have emerged, it may be helpful to review the various hypotheses that have been proposed from time to time to account for the behavior of this most complex and important group of substances.

It is not too much to say that none of the structure hypotheses is wholly satisfactory. Each provides an explanation of certain features of protein chemistry and, with a few exceptions, all have been, or are, the point of departure for stimulating research. The structure of proteins is, nevertheless, still an unsolved problem, and it is with the hope of stating this problem in clearer terms that the present paper is written.

I. EARLY SPECULATIONS. The term protein had its origin in the earliest attempt to explain the constitution of these substances. The word was first used by Mulder and, in order to understand exactly what he wished it to connote, it will be necessary to recall one or two of the important chemical events of the third decade of the last century. This was a period in which there was little agreement among chemists

[1] The expense of the preparation of this paper was shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

The authors wish to express their appreciation of suggestions and criticisms made by Prof. Lafayette B. Mendel of Yale University.

as to the formulation of organic compounds. While the conception of the chemical atom had been clearly defined by Berzelius there was no unifying principle by which molecular magnitudes could be established. Formulae were mainly written in terms of equivalents and an immense step in advance was made by Liebig and Wöhler (119) in 1832 in their investigation of the oil of bitter almonds (benzaldehyde) when they showed that a whole series of related compounds could be formulated in terms of one uniform group of atoms which they called the radical. Thus benzaldehyde was, in their notation, BzH_2, benzoic acid BzO_2, benzoyl chloride $BzCl_2$, etc. This observation gave rise to an enthusiastic search for other series of compounds that could be formulated in a similarly simple fashion and led to the clear conception of organic radicals which remains to the present day. It was only natural, therefore, for Mulder, who had become interested in the study of albuminous substances, to attempt to fit them into a scheme which had such signal success in other fields. He carried out analyses of fibrin, serum albumin, egg albumin, silk and other proteins, and found that they all had approximately the same ultimate composition which was represented, according to Berzelius' notation, as $2\ C_8H_{12}N_2+5\ O$, since it was necessary that the radical should not contain oxygen (135). Mulder believed that the oxidized radical was combined in the proteins with sulphur and phosphorus in the ratio of twenty-five to one sulphur and one phosphorus atom in fibrin, and of twenty-five to one sulphur and two phosphorus atoms in serum albumin. In the following year, as the result of further analyses (136), he suggested that the proteins are compounds of sulphur and phosphorus with an organic radical which he now wrote $C_{40}H_{62}N_{10}O_{12}$. This radical he called "protein" with the implication that it formed the basis of all albuminous substances, just as Liebig had spoken of "benzoyl," the radical or basis of benzoic acid. Fibrin and egg albumin were now written $\overline{Pr}_{20}PS_2$, and serum albumin $\overline{Pr}_{20}PS_4$, while the precipitates of proteins obtained with tannic acid, lead oxide and hydrochloric acid were formulated in a similar manner, since Mulder believed that a double decomposition occurred and the tannic acid or other reagent displaced the sulphur and phosphorus.

The precipitate obtained when a solution of fibrin in hydrochloric acid was treated with ammonium carbonate was, according to Mulder, the binoxide of protein. Liebig showed (118) that this material contained sulphur and also that other proteins, when precipitated in a similar manner, likewise contained sulphur. Further, Laskowski (115)

showed that Mulder's formulae were not always in strict accordance with his own analyses and that sulphur-free preparations of proteins could not be obtained. It was in vain for Mulder (137) to suggest that the sulphur of the protein was converted into potassium pentasulphide by treatment with alkali, and that, on acidification, free sulphur was precipitated along with the protein. This could not be demonstrated and Mulder's "protein theory" collapsed.

No further attempt to arrive at a notion of the constitution of proteins was made for many years. Liebig (117) asserted that fibrin, casein and albumin were of the same composition and that these, with vegetable fibrin (probably the glutelins of today), were the chief proteins in nature. This idea of identity permeates all of the work of Ritthausen and of others who devoted themselves to the preparation of proteins from various sources.

Schützenberger in 1875 (160, 161, 162, 163, 164) investigated the action of barium hydroxide upon various proteins and observed the formation of ammonia, carbon dioxide and oxalic acid. From the relative proportions of these products he drew the conclusion that urea groups and oxamide groups are pre-formed in the protein molecule. Habermann and Ehrenfeld (71) repeated Schützenberger's work in 1900 but were wholly unable to reconcile with this view the quantities of ammonia and carbon dioxide that they obtained by hydrolysis. Moreover they could not confirm the presence of oxalic acid in alkaline hydrolysates and pointed out numerous inconsistencies and sources of error in Schützenberger's work.

As is now known, Schützenberger's idea was partially correct. The guanidino group does occur in proteins and is the source of a part of the ammonia and carbon dioxide obtained on alkaline hydrolysis. In fact guanidine was actually prepared from proteins by oxidation with permanganate by Lossen (122) and urea was obtained by Drechsel in 1890 (51) by alkaline hydrolysis of "lysatine," a product of hydrolysis of proteins that was later shown to contain arginine. But the rest of Schützenberger's ideas were mere speculation and the experimental foundation of his one correct idea was wholly insufficient. It was simply a lucky guess.

II. "GROUP" AND "NUCLEUS" SPECULATIONS. During the latter half of the last century much attention was paid to the action of the proteolytic enzymes and, as information accumulated, an important attempt was made by Kühne and his pupils to arrive at an hypothesis of protein constitution which should be in harmony with the facts.

The so-called hemi- and anti-group hypothesis that he proposed was the foundation upon which practically all the physiological work and most of the chemical work on proteins of this period was based. That it is now practically forgotten is due largely to the unfortunate emphasis that was placed upon preparations which responded to none of the criteria applied by chemists for the recognition of pure homogeneous substances. With the advent of the better founded views of the present century these older ideas were discarded, but to the historically-minded they have much interest if for no reason other than the lesson they teach of the fate of speculations which are founded upon observations that are not sufficiently established or wholly understood. As will later appear there is, even at the present day, a situation in protein chemistry to which this lesson applies and it may therefore be profitable to spend a few moments in the consideration of ideas that are now extinct.

A. *The hemi- and anti-group hypothesis of Kühne.* Meissner (124, 125, 126, 127, 128) in 1859 was probably the first to observe that proteins, when exposed to the action of pepsin, were incompletely digested and that a product that resisted the further action of this enzyme could be isolated. These results were treated with skepticism or wholly disregarded until Kühne in 1876 (110) made the same observation and, further, showed that the pepsin-resistant substance was attacked by trypsin with the production of tyrosine, "leucine,"[2] and a complex substance which, in turn, resisted the further action of this enzyme. This behavior was supposed by Kühne to indicate the presence in the protein molecule of groups or radicals of two different types. Although proteins differed among themselves in details of composition, behavior and reactions, all of the simpler ones, at least, appeared to possess this feature in common. Kühne denoted that part of the protein molecule which resisted the action of enzymes the anti-group, while the part which was readily decomposed was the hemi-group. These ideas were for many years actively developed by Kühne and his distinguished pupils Chittenden and Neumeister. The fundamental principle of their investigations was the digestion of various proteins with enzymes, or with dilute acid, under definite conditions, followed by elaborate and

[2] The "leucine" of the older investigators was a white substance crystallizing in balls or nodules, figures of which may be found in some of the older textbooks. It was undoubtedly a complex mixture of amino-acids. Mulder, however, appears to have prepared a relatively pure specimen of leucine. He described it as consisting of gleaming white plates with a greasy feel which floated on water.

infinitely laborious attempts to separate the products of the digestion by means of salting out[3] with sodium chloride, magnesium sulphate or ammonium sulphate, under various conditions of temperature and reaction.

The results of this work may be summarized as follows (142, 38, 39). The action of pepsin upon proteins led first to the formation of syntonin which was then split into two different primary albumoses[4] called protalbumose and heteroalbumose. The former was soluble in water, the latter only in the presence of neutral salts, thereby resembling the native globulins. Further, on standing under water, or on drying, heteroalbumose frequently underwent a kind of denaturation becoming insoluble in neutral solvents. This alteration product was dysalbumose. The change could be partially reversed by solution in acids or alkalies. On neutralization a part of the dysalbumose remained in solution and thus regained the solubility of heteroalbumose.

Further action of pepsin upon the primary albumoses led to secondary albumoses (deuteroalbumoses) which were difficult to distinguish or to separate from each other; still further action led to the soluble peptones. The number of secondary albumose molecules produced from the two primary albumoses was uncertain since the molecular weight of none of these products was known.

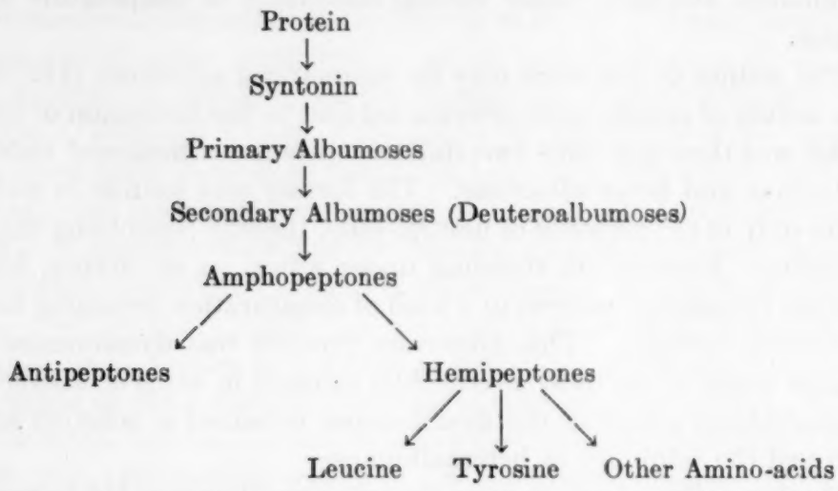
By tryptic digestion proteins were broken up into two kinds of peptones of which one kind, the hemipeptones,[5] were easily decomposed by the enzyme yielding "leucine" and tyrosine. The other kind, the antipeptones, could not be so decomposed. The two kinds were formed in approximately equal amount. Peptic digestion, which does not proceed far enough to set free amino-acids, did not separate the members of the hemi- and anti-groups from each other and peptones obtained by peptic digestion were therefore termed amphopeptones since they

[3] See, for example, Kühne and Chittenden (111). Many of Kühne and Chittenden's papers may be found translated in Studies from the Laboratory of Physiological Chemistry, Sheffield Scientific School of Yale University 1884–1888, 3 vols., New Haven, 1885, 1887, 1889.

[4] The term albumose or albuminose was suggested by Mialhe (130) in order to draw attention to the analogy between the production of soluble and diffusible substances from proteins by one type of enzyme and from complex carbohydrates by another. Both reactions were recognized as a form of hydration and the alteration of the protein was comparable to the change of insoluble starch into soluble sugar.

[5] Hemipeptone is not the antithesis of antipeptone. On treatment with trypsin approximately one-half of the protein was dissolved or decomposed, hence hemipeptone denoted the easily decomposed half.

yielded both hemi- and antipeptones on tryptic digestion. The sequence of events may be indicated as follows:

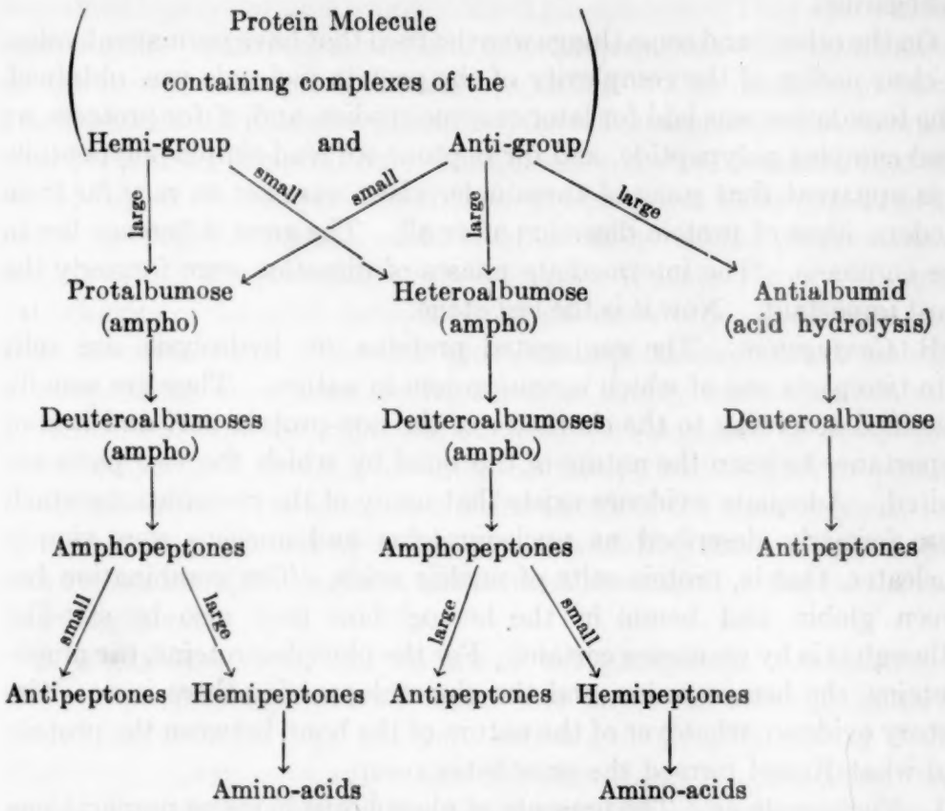


This is, however, the simplest expression of the situation; in practice further complications arose. The different amphopeptones obtained by peptic digestion did not appear to contain hemipeptones and antipeptones in equal proportions since the tryptic digestion of heteroproteoses indicated that these contained excess of antipeptones while the protalbumoses gave larger proportions of amino-acids. Furthermore, the action of dilute acid upon proteins led to the formation of an insoluble substance which appeared to be digested with difficulty by enzymes and to yield only antipeptones. This was therefore considered to represent a considerable part of the anti-group of the protein and received the name antialbumid. These various relations can perhaps be better appreciated from the scheme on page 399 founded upon Neumeister (143), which represents the decomposition of the protein.

The fundamental fact which gave rise to this elaborate system was the observation that peptones were diffusible. They were therefore regarded as the means by which proteins were conveyed from the intestine into the blood stream (86, 87, 88). It must be borne in mind that erepsin, the enzyme which is preëminently associated with the final stage of digestion, the splitting of the smaller groups or peptones into amino-acids, was not discovered until 1901 (45, 46). Moreover, amino-acids were not recognized in the blood until 1913 (1). Consequently peptones had previously been immensely important substances.

It is not within the province of this paper to discuss the extensive literature of the proteoses. For this the early textbooks, such as those

of Cohnheim, Neumeister or Gamgee, may be consulted. The essential point here is to discover the trend of thought of the time. The origin of the interest in peptones has been shown. Looking back upon the results it is now possible to assess their value.



The older investigators were dealing with mixtures of unknown composition which could not be separated into homogeneous definite substances. The various salting-out methods they employed for the separation of the products of protein digestion were not capable of giving results of great chemical significance, and, at the close of the century, the possiblities of Kühne's methods had been practically exhausted. Aside from ultimate analysis, no effort was made in the early days of the proteose period of protein chemistry to characterize preparations by their amino-acid composition or nitrogen distribution, although Hofmeister and his pupils did later attempt to obtain more precise characterizations. Little emphasis was placed upon amino-acids even when they were encountered. Neumeister, in 1897, when discussing the decomposition products of proteins, mentions tyrosine, leucine, and aspartic acid together with ammonia and hydrogen sulphide

as the regular digestion products, while glutaminic acid, lysine and arginine could be formed by the action of strong acids upon proteins. Glycocoll, alanine and serine receive only passing attention when silk proteins are mentioned; phenylalanine, histidine and cystine are wholly disregarded.

On the other hand some things were learned that have permanent value. A clear notion of the complexity of the protein molecule was obtained. The foundation was laid for later enzyme studies, and, if for proteose we read complex polypeptide, and for peptone we read simple polypeptide, it is apparent that some of these older views were not so very far from modern ideas of protein digestion after all. The great difference lies in the emphasis. The intermediate phases of digestion were formerly the most important. Now it is the last stage.

B. *Conjugation.* The conjugated proteins, on hydrolysis, are split into two parts one of which is non-protein in nature. They are usually classified according to the character of the non-protein part and it is of importance to learn the nature of the bond by which the two parts are united. Adequate evidence exists that many of the preparations which were formerly described as nucleoproteins and nucleins were simply nucleates, that is, protein salts of nucleic acids. The combination between globin and hemin in the hemoglobins may also be salt-like although it is by no means certain. For the phosphoproteins, the mucoproteins, the hemocyanins, and the glucosidoproteins there is no satisfactory evidence whatever of the nature of the bond between the protein and what Kossel termed the prosthetic group.

1. *Nucleoproteins.* The presence of phosphorus in many preparations of proteins was recognized very early and two general types of these more complex substances were distinguished. The nucleoalbumins contained small proportions of phosphorus and, on digestion with pepsin, yielded a small amount of so-called paranuclein. Casein is an example of this type. Substances of the other type were proteids, that is, compounds of true protein with a phosphoric acid-containing complex. These, on digestion, gave an insoluble nuclein which contained a high proportion of phosphoric acid and from which, on hydrolysis with acids, the so-called nuclein bases, adenine, guanine, xanthine and hypoxanthine, could be isolated. Pancreatic digestion of the nucleins yielded protein decomposition products and nucleic acid, a substance which has attracted a great deal of attention from the early days of protein chemistry to the present.

It is unnecessary and would be tedious to discuss the confused lit-

erature dealing with the older views of the nature of the nucleoproteins. The relationship of the nucleic acid to the protein was first clearly pointed out by Osborne and Campbell (145). These authors had found that preparations of the proteins of the wheat embryo could be made which contained highly variable proportions of phosphorus. They also yielded nucleic acid. When the analyses of these preparations were recalculated on the nucleic acid-free basis, the proportion of this constituent being assumed from the phosphorus content, the residual protein was shown to have a constant composition. Consequently it was concluded that the variability of the composition of these products was to be explained by assuming that the protein could combine with varying proportions of nucleic acid. The nature of the combination appeared, from the chemical behavior of the substances, to be of the same order as the combinations between other vegetable globulins and mineral acids and the nucleoproteins were therefore regarded as simply nucleic acid salts of protein. For similar reasons the nucleins were regarded as compounds of protein, or its hydrolytic products, containing a still higher proportion of nucleic acid. This conception of the nature of the nucleoproteins and of the nucleins brought order out of a bewildering chaos of speculation and experimental findings and subsequent investigation has only served to strengthen it.

2. *Phosphoproteins.* The nature of the phosphorus combination in the phosphoproteins, such as casein, and in certain other phosphorus-containing proteins (egg albumin, serum globulins) is still in doubt. There is no question whatever that the phosphorus is a constituent part of the molecule, but beyond the fact that it is combined in some way as phosphoric acid, possibly as an ester, very little is known (155, 156, 157, 169).[6]

3. *Mucoproteins.* Another type of conjugated protein which has been long recognized is that which contains a carbohydrate group. Of these the mucins have attracted much attention on account of their wide-

[6] Since the above was written Rimington's papers on the phosphorus of casein (Biochem. Journ., 1927, xxi, 1179, 1187) have appeared. He has isolated a polypeptide from casein digests which is constituted entirely of oxyamino-acids and contains phosphoric acid united as an ester upon the hydroxy groups. At least one-half of the total phosphorus of the casein was definitely accounted for in this substance. There seems no reason to doubt, therefore, that the phosphorus of casein, and probably also that of other phosphoproteins, is combined as an ester of phosphoric acid upon hydroxy amino-acids and, further, that the greater part of the phosphoric acid is localized in a relatively small part of the protein molecule.

spread occurrence in the animal body (72, 73, 132). Krukenberg (109) in 1884 investigated the so-called chondroitic acid that had been obtained from tendons by Bödeker in 1854 (66), and Schmiedeberg in 1891 (159) showed that this substance was a complex compound of sulphuric acid which he called chondroitin sulphuric acid. Its nature has been thoroughly investigated by Levene (116) and its relationships to the amino sugars settled; but the relationship of the chondroitin sulphuric acid to the protein moiety of the conjugated protein is still undetermined, although Schmiedeberg suggested that this was probably analogous to a salt. He compared the chondroitin sulphuric acid to tannic acid and believed that the insoluble precipitate obtained when solutions of chondroitin sulphuric acid and gelatin were mixed was of the same nature as the "glutinchondrin" that could be extracted from tendons. Takahata (176) has recently verified this old observation of Schmiedeberg and is convinced that the relationships in the mucins are really salt-like. His experiments in support of this contention are, however, unconvincing. The formation of an insoluble precipitate when collagen is treated with chondroitin sulphuric acid may be evidence of salt formation, but it does not show that the insoluble substance has the same constitution as a true chondroprotein. The question is still open.

4. *Chromoproteins.* The chromoproteins provide a further example of an obscure type of combination between a protein and a second or prosthetic group. Although the physical and chemical properties of the best known chromoprotein, hemoglobin, and its various transformation products have been studied with great care, the relation between the iron-containing pigment, hemin, which can be isolated in crystalline form, and the protein globin has not yet been made entirely clear.

Anson and Mirsky (19, 20, 21, 22) have recently assigned the designation hem, to the base, the hydrochloride of which is hemin, and believe that this substance is a respiratory pigment that is universally distributed in nature. It is thought to be "in equilibrium with" various substances such as globin, thereby forming hemochromogens. The hemochromogen formed from hem and globin is supposed to polymerize, under proper conditions of hydrogen ion concentration, to hemoglobin which contains four such units. The oxidized form of hemochromogen is hematin which is believed to contain one globin molecule. Treatment with acid decomposes this association and liberates hemin and globin. All of these compounds, according to Anson and Mirsky, may be connected by equilibrium equations and the position of the

equilibrium may be altered by changes in the concentration of the reacting substances or of the hydrogen ion.

Whether Anson and Mirsky's views of the nature of hemochromogen and hematin are correct or not remains to be demonstrated but their suggestion that the pigment is "in equilibrium with" the protein in hemoglobin implies a type of combination that may be either salt-like or ester-like. Steudel and Peiser (172) have stated their conviction that the union is salt-like on account of the extreme ease with which the pigment may be liberated from its combination by acid. They failed, however, to prepare a reconstituted hemoglobin owing to the denaturation of the globin which occurred when hemoglobin was treated with acid. Hill and Holden (85) have, however, been able to prepare an undenatured globin practically free from methemoglobin which is entirely soluble over the range pH 5–10 and which, on treatment in faintly alkaline solution with hemin, gives a solution that shows the typical absorption bands of methemoglobin. This material could then be converted by appropriate treatment into products that gave absorption bands very close to those of hemoglobin and oxyhemoglobin. If spectroscopic evidence may be accepted as proof of the reconstitution of hemoglobin from its constituent parts, the behavior described by Hill and Holden is closely analogous to that of an acid and a base in salt formation. Further, this view is entirely consistent with our present knowledge of the relations between other proteins and dyes (37).

The presence of copper in the blood of *Octopus vulgaris* and *Helix pomatia* was discovered in 1847 by Harless (75) and the blue copper-containing protein of octopus blood, hemocyanin, was first obtained by Fredericq in 1878 (69). Henze prepared this protein in crystalline form in 1901 (79, 81) and investigated its properties. He was unable to verify Fredericq's statement that a copper-containing substance analogous to hemin could be separated from the protein by treatment with acid, but he fully supported Fredericq's observation that hemocyanin played the part of a respiratory protein in the blood of octopus.

Copper-containing proteins have since been frequently studied and have been shown to occur in numerous gastropods and arthropods. An excellent review of their properties was given by Quagliariello in 1923 (152) and the physiological functions of some of them have been recently investigated by Redfield, Coolidge and Hurd (154) with every attention to the modern technique of gas analysis. These authors showed that the hemocyanins play the same part in the transport of oxygen and carbon dioxide as does hemoglobin. Further work (153)

has shown that the combining ratio of oxygen and copper is such that, in a large series of these proteins, one atom of copper combines with one of oxygen.

In spite of much investigation the nature of the linkage of the copper in the hemocyanins is still a matter of debate. The similarity in the behavior towards oxygen suggests that they possess an essentially identical copper group, although the protein part appears to vary. The difference between the hemocyanin of Limulus and that of Octopus is especially marked, the copper content of the former being 0.173 per cent and of the latter 0.38 per cent (153). Pure hemocyanin, dissolved in water, gives no reaction whatever for copper ion with potassium ferrocyanide or hydrogen sulphide until the solution has been acidified, and it is therefore believed that the copper is combined in some type of complex compound in which the properties of the copper ion are wholly masked. That proteins can combine with copper even in relatively large proportions has been shown by Osborne and Leavenworth (147) and the capacity of copper to combine with peptides has been investigated by Kober and Sugiura (100, 101) but whether or not these copper "salts" have any relation to the hemocyanins is wholly unknown.

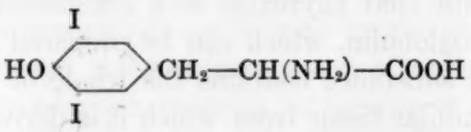
Still other types of chromoproteins are found in numerous marine algae. Kylin (113, 114) has carried out extensive investigations of these substances, especially the phycoerythrin and phycocyanin of *Ceramium rubrum*. These two choromoproteins, as well as similar substances from many other algae, were prepared in the crystalline form and their protein nature demonstrated conclusively. The pigment complex is liberated from the protein by treatment with dilute acid and it may then be extracted by such solvents as amyl alcohol or ether. Nothing is known either of the chemical nature of the pigment or of the type of combination between it and the protein, but the general behavior of these chromoproteins with respect to the linkage of the pigment moiety recalls that of hemoglobin.

5. *Iodoproteins.* While the iodoproteins can scarcely be classified as conjugated proteins they provide an example of a unique type of molecular structure in the protein field, and it is one, moreover, that has assumed great importance in view of the recent investigations of thyroxine.

Vogel, as early as 1848 (184), had noticed that certain marine animals contained iodine in organic combination. Drechsel in 1896 obtained an iodine-containing compound (52) which he named iodogorgoic acid, by the alkaline hydrolysis of the horny skeleton of *Gorgonia Cavolinii*, a coral that had been found to possess an extraordinarily high content of

iodine. Henze (80) subsequently investigated this substance and pointed out that it should be regarded as an aromatic derivative since it gave the xanthoproteic reaction. In fact Hundeshagen had already suggested (52) that the iodine in these curious proteins was probably present as a substituent in the benzene ring of tyrosine.

In 1905 Wheeler and Jamieson (191) synthesized 2-5, diiodotyrosine by the iodation of l-tyrosine obtained from proteins and became convinced that their product (I)



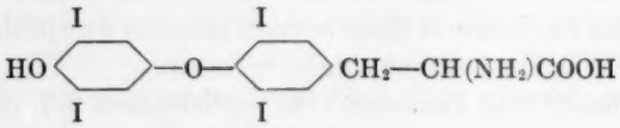
I. l,2-5 diiodotyrosine (iodogorgoic acid)

was identical with the substance previously obtained by Drechsel. Henze (82) repeated their work and could not reconcile the solubility and crystalline form of the synthetic substance with iodogorgoic acid, samples of which he had in hand. Inasmuch as the natural product had been obtained by alkaline hydrolysis of the protein it appeared probable that racemization had occurred. Henze therefore prepared racemic tyrosine, iodated it and showed that racemic 2-5, diiodotyrosine was identical with iodogorgoic acid in every respect. It is, however, more than likely that the substance actually present in the original protein is optically active and of the same configuration as l-tyrosine. Consequently the substance synthesized by Wheeler and Jamieson probably represents the real protein constituent.

The presence of iodogorgoic acid in the horny skeleton of the common sponge was later shown by Wheeler and Mendel (192) and it has recently been isolated from the Gorgonian coral *Plexaura flexuosa* by Mendel and Sugimoto (129). It is highly probable that this substance occurs in the numerous iodine-containing keratins that are found in similar marine animals.

The modern interest in iodine-containing proteins had its origin in Baumann's discovery in 1895 (23) that iodine is a normal constituent of thyroid gland tissue and the subsequent successful attempts to correlate the physiological action of thyroid tissue with its iodine content (91, 92). The isolation of the active constituent of the thyroid gland, thyroxine, by Kendall (99) in 1919 was followed by the demonstration by Harrington (76) that thyroxine is allied to tyrosine and is an α-amino-acid. In 1926 Harrington and Barger synthesized thyroxine (77) and it is ap-

parent from formula II that this substance bears a close relationship to iodogorgoic acid.



II. Thyroxine

There is little doubt that thyroxine is a constituent of the peculiar protein, iodo-thyreoglobulin, which can be prepared from the thyroid gland (144). This substance contains the whole of the physiological potency of the glandular tissue from which it is derived, and thyroxine is obtained from whole glands only after a prolonged alkaline hydrolysis closely analogous to that employed in the preparation of iodogorgoic acid. There seems no reason, therefore, to exclude iodogorgoic acid and thyroxine from the list of well established protein amino-acids.

C. *Kossel's protamine nucleus hypothesis.* The discovery by Miescher in 1874 of a basic substance in the sperm of salmon which he named protamine (131) (see also Piccard (151)) attracted little attention until 1894, when Kossel began his investigations of this and similar bases (103). Kossel found that these substances, on hydrolysis, yielded large proportions of arginine; in one of them, sturine, a new base which he named histidine, was discovered. At the same time Hedin (78) obtained a base from several proteins that proved to be identical with this new compound. The relationships of these curious substances became clearer and further investigations at once (104, 105, 108, 112, 134) showed that the protamines, the original term applied to the first representative by Miescher having been extended to denote the entire class, consisted essentially of amino-acids, could be digested by trypsin and were therefore allied to the proteins. Kossel advanced the view that they should be regarded as the simplest representatives of this class of substances.

It has been pointed out that the proteins were then looked upon as composed of a number of differently constituted parts that could be separated, after hydrolysis by means of enzymes or dilute acids, by the elaborate salting-out technique of Kühne and Chittenden, of Pick and of others. Nothing had been found, however, which led to a clear comprehension of the nature and composition of these parts. Kossel attacked the problem of constitution from another point of view and showed that protein-like substances existed which had a relatively simple

constitution, containing large proportions of only a few amino-acids among which the basic amino-acids, in particular arginine, preponderated. It was a logical extension of previous views for him to suggest that the more complex proteins might be built around a central "nucleus" (106) by the addition of other groups. The central nucleus must contain those amino-acids which are common to all proteins and the available data pointed to arginine as the most important of these. A table, part of which is reproduced below, was given by Kossel to illustrate the wide variation in arginine content of various proteins. He suggested that the protamines are formed by the attachment of a few other amino-acids to a complex compound of arginine of unspecified nature. The more complex proteins in turn were to be regarded as built up by the attachment of amino-acids and larger structure units to a "protamine nucleus."

TABLE A

The arginine content of various proteins

	ARGININE
	per cent
Salmine	84.3
Clupeine	82.2
Sturine	58.2
Edestin	14.07
Gelatine	9.3
Casein	4.8
Gliadin	2.75
Zein	1.82

This suggestion of Kossel did not meet with favor. The rapid development of the polypeptide hypothesis, founded on experimental facts rather than on speculation, completely overshadowed it. Morever, the low proportion of bases in silk and certain vegetable proteins did not support this view. Under the influence of Fischer, clearer conceptions of structure were taking the place of the somewhat confused and indefinite ideas of the older type; but, in spite of this, there was one long continued and serious attempt to get at the nature of the hypothetical "nucleus" of the protein molecule. This was Siegfried's investigations of the kyrines.

D. *Kyrines.* Siegfried (165, 166), from 1903 for a period of several years, devoted much study to the problem of isolating products of a

definite nature from partially hydrolyzed proteins (fibrin, gelatin, casein, etc.) and succeeded in obtaining a series of basic substances which, on further hydrolysis, yielded arginine, lysine and glutaminic acid, in approximately molecular proportions. A few of them appeared to contain either glycocoll or alanine in addition. The close resembance of these preparations to each other in properties and composition led him to suggest the generic name kyrine for them with the implication that they were the kernel, or nucleus, of the molecular structure of the proteins from which they were derived. This view was founded upon the resistance to hydrolysis of the kyrines under the conditions employed in their preparation and is therefore an extension of the older ideas of Kühne. The kyrine idea also fitted in well with Kossel's protamine nucleus hypothesis inasmuch as the kyrines are, like the protamines, strongly basic substances.

Neither of these suggestions contained that essential feature of a useful hypothesis, an indication of the path along which future investigation must proceed. Active investigation was, however, stimulated by the polypeptide hypothesis of Hofmeister and Fischer and these other views receded farther and farther into the background, as time went on, until they have now become all but forgotten. They illustrate, however, the influence of the thought of the last century and the attempts to graft the newer knowledge upon the old.

III. THE FUNDAMENTAL FACTS OF PROTEIN CHEMISTRY. The object of speculation upon the structure of proteins is to obtain a mental picture of the molecular configuration of these substances which will explain their chemical behavior and thereby effect economy of thought. It will, perhaps, be of value, therefore, to indicate briefly the facts of protein chemistry which must be explained, in whole or in part, by any useful hypothesis of their constitution.

The two most fundamental facts are, of course, that proteins, on hydrolysis with acids or alkalies, yield a large variety of α-amino-acids and, secondly, that proteins are, in general, hydrolyzed by the proteolytic enzymes with the ultimate production of these same α-amino-acids. The amino-acids belong to three distinct types, those containing an excess of basic groups, those containing an excess of acidic groups and those which are essentially neutral.

The basic amino-acids are,

Arginine
Histidine
Lysine

These are found in practically all native proteins, zein from maize being the most noteworthy exception as lysine has not hitherto been obtained from it.

The acidic amino-acids are,

Aspartic acid
Glutaminic acid
Oxyglutaminic acid

Of these the first two have been found in all proteins that have been carefully studied. Oxyglutaminic acid has been found in most of those which have been examined for it but, as yet, the list is short.

The essentially neutral amino-acids are,[7]

Glycocoll	Cystine
Alanine	Proline
Valine	Oxyproline
Leucine	Tryptophane
Isoleucine	Phenylalanine
Serine	Tyrosine

and to these it is probably justifiable to add the substituted tyrosine derivatives, found only in certain unique proteins,

Iodogorgoic acid
Thyroxine

A number of other substances have been described from time to time as protein constituents, but none of these has been so clearly characterized as to warrant its inclusion in a list of well established amino-acid derivatives of proteins.

Most proteins yield appreciable proportions of each of these amino-acids. An adequate speculation upon the constitution of proteins must therefore take account of the fact that amino-acids of all three types are

[7] J. H. Mueller has (Proc. Soc. Exper. Biol. and Med., 1921–22, xix, 161; Journ. Biol. Chem., 1923, lvi, 157) described a sulphur containing amino-acid which was isolated from several proteins and the analysis of which led to the empirical formula $C_5H_{11}SNO_2$. Barger and Coyne have recently (personal communication) synthesized the substance $CH_3-S-CH_2-CH_2-CH(NH_2)-COOH$, and have shown that it is identical with Mueller's amino-acid and with preparations obtained from proteins by the method described by him. Mueller has agreed to Barger's suggestion that the new amino-acid should be named *Methionine*, and this substance should now be added to the list of well established neutral amino-acids.

present and must also consider the peculiar chemical nature of many of the neutral amino-acids.

In addition to the amino-acids, variable proportions of ammonia, a small proportion of carbon dioxide and, usually, traces of free sulphur are produced by acid hydrolysis.

The native proteins are amphoteric substances although the maximal acid and base binding capacity of most of them is small. The presence of small proportions of free amino groups has been detected in many proteins and that of small proportions of free carboxyl is inferred from the capacity to form soluble alkali salts. These quantities, however, bear no relation to the relatively enormously greater proportions of amino and carboxyl groups present after the protein has been subjected to hydrolysis. Obviously, therefore, the union of the amino-acids is effected in some way which locks up the greater part of the amino and carboxyl groups. Further, as nearly as can be found by present methods of investigation, amino and carboxyl groups are produced during hydrolysis, whether by acids or by enzymes, at the same rate in relatively equivalent amounts.

Proteolytic enzymes of three general types have been recognized which, from their analogy to the enzymes of the alimentary tract, are usually classified as pepsins, trypsins and erepsins. Regarding the action of pepsin upon proteins little is known except that it liberates amino and carboxyl groups usually to the extent of 15 to 20 per cent of the total amount (68) that can be obtained by complete hydrolysis. There is, at present, some debate whether these are formed in the early stages of digestion in strictly equivalent amounts. The nature of the chemical bonds that are broken by this enzyme is wholly unknown.[8] Free amino acids have never been found in peptic digests prepared under proper conditions and most observations indicate that the general effect of this enzyme is to decompose the molecule into relatively large fragments.

Although trypsin and erepsin have been classified as peptide splitting enzymes, the recent work of Waldschmidt-Leitz (see below) has rendered this less certain with respect to trypsin. This enzyme acting upon a peptic digest usually carries the hydrolysis up to 70 per cent of completion (68); when acting upon native protein up to 50 per cent, and liberates considerable proportions of free amino-acids. According to Waldschmidt-Leitz, adequately purified trypsin does not attack simple peptides and the nature of its real substrate is therefore somewhat in doubt. Erepsin, however, is thoroughly established as a peptidase. It

[8] Since this was written Sörensen and Katschioni-Walther have shown (Zeitschr. physiol. Chem., 1928, clxxiv, 251) that amino and carboxyl groups appear in strictly equivalent proportions during peptic hydrolysis.

is known to carry the hydrolysis of tryptic digests practically to completion and to hydrolyze synthetic peptides containing the natural isomers of the protein amino-acids.

One of the most obscure reactions of proteins is that of the denaturation, or loss of solubility, under the influence of heat or, in many cases, alcohol. The best known example, the coagulation of egg albumin, is fairly typical, but other proteins vary not only in the temperature at which denaturation occurs but also in the velocity of the reaction and the completeness with which denaturation can be brought about. Some of the vegetable globulins are only incompletely denatured even by boiling water.

The effect of alcohol is equally difficult to interpret. Albumins are promptly denatured by it, but many vegetable globulins are only partly altered, while a solution of the prolamin zein in 90 per cent alcohol will deposit only small amounts of insoluble denatured protein even on long standing.

The problem of the solubility of proteins is still filled with obscurity. Some proteins are readily soluble in water; others, for example those of skin and horn, are among the most insoluble of substances. The solubility relationships of the globulins are especially curious. These proteins are insoluble in water but readily dissolve in dilute solutions of neutral salts.

Still another point must be considered by those who could propose an hypothesis of protein structure. Leaving aside the protamines which, although they have been considered the simplest representatives of the class of proteins, present several clearly defined differences, there are good reasons for regarding egg albumin as possessing one of the smallest of true protein molecules. Ultra filtration experiments (40) have shown that filters could be constructed through which this protein would pass but which retained the other proteins that were investigated. Be this as it may, there are several independent and trustworthy lines of evidence that point unmistakably to a molecular weight of the order 34,000 for this protein (44, 167, 169, 174). A careful consideration of the iron and sulphur content of hemoglobins from different animal species indicates that the molecules of these proteins cannot be smaller than egg albumin and the evidence is in favor of a figure of the order 68,000 (17, 173, 175). The serum proteins are almost certainly much larger. Thus it is relatively certain that many of the soluble proteins possess truly gigantic molecules.

These essential facts of protein chemistry have been borne in mind in the following discussion of various suggestions that have been made to

explain the structure of proteins. A somewhat critical attitude has been adopted because the present widespread interest in proteins has occasionally led to the over enthusiastic and somewhat premature acceptance of views, some of which are neither well grounded nor in strict agreement with all that is now known.

IV. THE "AMIDE-NITROGEN" OF PROTEINS. The first attempt to account for the presence of ammonia among the products of hydrolysis of proteins was made by Nasse in 1872 (139, 140, 141). He observed that the amount of ammonia produced by alkaline hydrolysis of proteins exceeded that produced by acid hydrolysis and that the liberation of ammonia by alkalies was at first rapid but subsequently very slow. This indicated the presence of two ammonia-yielding groups in proteins. He pointed out that the nitrogen of acid amides and of nitriles is readily

TABLE B

The amide nitrogen of proteins

PROTEIN	AMMONIA CALCULATED	AMMONIA OBSERVED	DIFFERENCE
	per cent	*per cent*	
Edestin	2.19	2.28	−0.09
Excelsin	1.99	1.80	+0.19
Amandin	3.36	3.70	−0.34
Legumin (vetch)	2.27	2.16	+0.11
Phaseolin	2.35	2.06	+0.29
Glutenin	2.83	4.01	−1.18
Gliadin	4.39	5.11	−0.72
Zein	2.29	3.61	−1.32
Casein	1.38	1.61	−0.23

liberated as ammonia when they are subjected to acid hydrolysis; amino-acids under these conditions are decomposed very slowly, while such substances as asparagine and uric acid give off a part of their nitrogen readily and the rest slowly, if at all. Judging by analogy, therefore, it appeared probable that the "loosely bound nitrogen" of proteins was combined in a form resembling the nitrogen of an acid amide or of urea. As a result of this well reasoned speculation the "loosely bound nitrogen" of Nasse entered into the literature as "amide nitrogen" by which term it has since been designated.

For many years no importance seems to have been attached to the amide nitrogen of proteins although Fleurent in 1893 (67) suggested that, inasmuch as wheat gluten yields large amounts of glutaminic acid and of

ammonia on hydrolysis, it was reasonable to suppose that the ammonia was combined in the protein as the amide of this acid. This suggestion attracted little, if any, attention.

In 1906 Osborne and Gilbert (146) determined the proportions of glutaminic and aspartic acids in a large number of proteins and pointed out a parallelism between the amount of ammonia obtained on hydrolysis from each of them and the yields of these acids. Two years later Osborne, Leavenworth and Brautlecht (148), assuming that one carboxyl of the dibasic amino acids contained in the protein was present as an amide group, calculated the proportion of ammonia which should then be liberated by hydrolysis and compared this with the actual yield. Some of the calculations are shown in Table B.

The analytical figures indicate a very close agreement in certain cases with the requirements of the hypothesis. In other instances, notably the cereal proteins, the discrepancy is large. Nevertheless this calculation further supported the views of Nasse.

At the present time there is less inclination to emphasize this evidence. In the first place the discovery of oxyglutaminic acid by Dakin in 1918 (48) provided a new source of carboxyl groups which must occur in may proteins, and, secondly, it is now known that the methods formerly employed to estimate aspartic and glutaminic acids were not sufficiently accurate for such a calculation to have the quantitative value that was formerly attributed to it (95).

When the results of the more accurate modern methods of analysis are considered in conjunction with measurements of the base-binding capacity of proteins, strong support is, nevertheless, furnished to the hypothesis of amide groups (41). The results of the analysis of zein, the prolamin from maize, are a striking example. Cohn and Berggren, making the same assumptions as Osborne, Leavenworth and Brautlecht, calculated that there should be 30.1×10^{-5} mols of free carboxyl per gram of zein. The measurement of the actual maximal base binding capacity gave 30×10^{-5} mols per gram. Gelatin and casein, which also have been carefully analyzed, likewise gave a close agreement between the calculated free carboxyl and that found.

Thierfelder and von Cramm in 1919 (177) made an interesting attempt to confirm the presence of amide groups in proteins by comparing the extent of hydrolysis, under certain conditions, of dipeptides of glutamine, the half-amide of glutaminic acid, with that of wheat gliadin, a protein that yields an extraordinarily large proportion of glutaminic acid on hydrolysis. They found that the proportion of the amide bonds set free

from the peptides corresponded very closely to that set free from gliadin and drew the conclusion that their data "further support the view that glutamine is contained in the protein molecule."

Osborne and Nolan (150) also have contributed evidence of an indirect nature. Since the amide nitrogen of gliadin could be set free by boiling with dilute acid, it was possible to titrate the reaction mixture after hydrolysis and, by taking account of all the acid present, together with the liberated ammonia and the added sodium hydroxide, to show that an acidity had been developed which was quite closely equivalent to the ammonia.

Unfortunately the evidence provided by enzyme experiments is not decisive. Hunter and Smith (93) studied the rate at which ammonia is split off from several proteins by trypsin and drew the conclusion that trypsin either has two functions, peptide splitting and amide splitting, or else that the preparation they employed contained two enzymes, a peptidase and an amidase. There is, however, a possibility which has been recently emphasized by Grover and Chibnall (70), namely, that the amide bond is not essentially different from the substituted amide (peptide) bond. Consequently asparagine may be regarded as a dipeptide, and the fact that it is hydrolyzed by peptide-splitting enzymes, such as plant erepsin, need not be surprising. The observation of Waldschmidt-Leitz, Grassmann and Schäffner (186), who found that leucine amide is readily hydrolyzed by pig erepsin, supports this view.

The available evidence, therefore, is distinctly in favor of the view that a large proportion of the dibasic acid groups are present as amides in the protein molecule. Moreover there is no conflicting evidence.

Before leaving the subject of amide nitrogen it is necessary to emphasize a point which is sometimes disregarded. Although it is highly probable that the greater part of the ammonia produced when proteins are boiled with acid has its origin in amide groups, there is little doubt that a small part has some other origin. This may be illustrated by Vickery's study of the rate at which the ammonia is set free from gliadin by acids of different concentration (183). Approximately 25 per cent of the nitrogen of this protein was promptly liberated as ammonia by boiling with comparatively dilute acid. Long continued boiling liberated a further 0.5 per cent at a very slow and apparently steady rate, and there was no reason to assume that the end of the process would have been attained within a reasonable length of time. This small additional quantity of ammonia has its origin in secondary decomposition of amino-acids. It is evident, therefore, that an ordinary determi-

nation of the "amide nitrogen" of a protein is not a precise measure of the amide groups. It merely sets an outside limit although this is usually close enough for most purposes.

It will be recalled that Nasse observed that alkalies liberate more ammonia from a protein than do acids. As was pointed out by Osborne, Leavenworth and Brautlecht, this excess is largely due to the secondary decomposition of arginine. In the study of the hydrolysis of gliadin already mentioned it was found that three separate phases of the hydrolysis of proteins by alkalies could be distinguished inasmuch as the liberation of ammonia proceeded at three different rates. The preliminary rapid formation of ammonia could be readily explained as the hydrolysis of amide groups. This was followed by a slower but quite steady evolution of ammonia which probably arose from the decomposition of the guanidino group of arginine. At the completion of these two reactions a very much slower production of ammonia was discernible which was apparently due to other secondary decompositions, possibly of cystine and tryptophane. These three reactions were, of course, concurrent but became evident as they successively terminated.

V. The peptide hypothesis. Hydrolysis by means of relatively concentrated boiling acids has been by far the most useful of the numerous methods that have been employed to decompose proteins and obtain products from which some notion of the constitution of the molecule might be derived. The formation of amino-acids by this means was early recognized, and it became of the greatest importance to discover how these substances were combined with each other in the proteins. To Hofmeister and to Fischer belongs the credit of having independently arrived at a probable explanation. The publication of Hofmeister's paper (89) on the structure of proteins in 1902 and, at about the same time, the appearance of a series of papers from Fischer and his collaborators (56, 58, 59, 65) dealing with the synthesis of polypeptides, and of the paper describing the ester method for the analysis of proteins (55), were probably the most important events in the whole history of protein chemistry. They at once clarified the situation and indicated the direction in which further progress was to be made.

Hofmeister discussed the various ways in which amino-acids might be supposed to be linked to each other in the protein. A direct union of carbon atoms could be ruled out as, in this case, the decomposition of proteins into definite units by acids, alkalies, or, in particular, by enzymes would be impossible to explain. A union of two carbon chains through an oxygen atom or atoms, that is an ether, ester or anhydride linkage,

might also be dismissed as, if this combination occurred to any considerable extent, hydroxy-amino-acids should be much more common among the products of hydrolysis of proteins than they have been found to be. Moreover, since this mode of combination leaves the amino groups free, a substance so constituted should be strongly basic in character. The most probable mode of union of amino-acids was therefore a combination through a nitrogen atom. Three possibilities were suggested, (1) $-CH_2-NH-CH_2-$; (2) $-CH_2-NH-CO$; (3) $-CH_2-NH-C(NH)-$. The first of these occurs in proline and oxyproline and is stable. Moreover, as it leaves carboxyl groups out of account, a compound of this type formed from amino-acids would be predominantly acid in nature. The third type occurs in only one of the protein amino-acids, arginine. It is stable to acid hydrolysis and may therefore be dismissed as a mode of union of amino-acids. Many considerations point to the second scheme as an actual linkage between amino-acids in proteins. Hofmeister enumerated the following:

1. Native protein itself contains very little amino nitrogen, but the end products of protein hydrolysis contain large amounts. The peptide bond type of union (as it was named by Fischer) readily accounts for this.

2. The biuret reaction is given by many substances which contain this group and this reaction is characteristic of proteins and their decomposition products, the proteoses. It disappears on complete hydrolysis. This strongly suggests the presence of the peptide bond in proteins and in their partial hydrolysis products.

3. A number of condensation products of amino-acids have been prepared which contain this group. Many of these give the biuret reaction.

4. The peptide union is also encountered in nature as, for example, in hippuric acid.

In addition to the reasons advanced by Hofmeister for believing that the peptide bond occurs in proteins, others which are even more striking can now be suggested.

5. The synthetic polypeptides obtained by Fischer from the natural isomers of optically active amino-acids are hydrolyzed by the enzymes of the digestive tract.

6. Polypeptides have frequently been found among the products of incomplete hydrolysis of proteins.

7. During the hydrolysis of proteins, whether by acids or enzymes, amino groups and carboxyl groups are progressively liberated at an approximately equal rate (74).

8. Hydrolysis of proteins occurs without material change in the hydrogen ion concentration of the solution. This is consistent with the view that equivalent amounts of amino and carboxyl groups are thereby produced.

9. Pepsin alone liberates as a rule about 20 per cent of the total amount of amino nitrogen which can be obtained by the complete hydrolysis of a protein. Erepsin, acting on a peptic digest can liberate as much as 70 per cent more (68). Since there is every reason to believe that the latter enzyme acts only upon peptide bonds it is obvious that by far the greater part of the total possible amino nitrogen of a protein has its origin in such bonds.

Taken together this body of evidence is as nearly conclusive proof that the peptide bond occurs to a very considerable extent in proteins as could be wished. There are, however, certain facts which remain unexplained upon the view that the protein is essentially a single large polypeptide.

In the first place the nature of the bonds that are attacked by pepsin is unknown. It has long been thought that this enzyme acts in such a way as to break the protein molecule into a number of large fragments and the recent work of Waldschmidt-Leitz lends further support to this view. Amino-acids are not liberated from the protein by it. It is reasonably certain, moreover, that this fragmentation is accompanied by the production of equivalent amounts of carboxyl and amino groups. Steudel (170, 171) is of the opinion that the number of carboxyl groups set free in the early stages of peptic digestion is somewhat in excess of the amino groups, but this contention is not supported by Waldschmidt-Leitz and Simons (190). Pepsin, like erepsin, attacks bonds in some way analogous to the peptide bond, but pepsin has never been found to have an effect upon any synthetic model substance whether it contained a peptide bond or not.

Secondly, it has not hitherto been possible adequately to account for the denaturation of proteins by heat upon any hypothesis of their structure. Sörensen (168) has shown that the denaturation of egg albumin by alcohol takes place without loss of nitrogen but that it very probably involves the loss of water from the molecule. This implies some sort of condensation reaction.

Further it is difficult to understand the insolubility in water of many proteins, particularly the albuminoids or keratins, upon the polypeptide view. Obviously some other type of union or some modification of the peptide bond must be invoked to explain these difficulties.

Although the polypeptide hypothesis explains a great many of the peculiarities of protein behavior, and the peptide bond is doubtless the most important mode of combination of amino-acids in proteins, Fischer specifically stated that other types of combination must be considered as possibilities (61). The diketopiperazine ring may occur and it is also highly probable that the hydroxyl groups of the oxyamino-acids play some part in the structure. Some of the recent attempts to expand these suggestions of Fischer are dealt with in the next section.

The effect of a well wrought theoretical conception of the structure of proteins upon science was immense. Kossel had already developed his method for the determination of the basic constituents of proteins and was occupied with the investigation of the protamines which he regarded as the simplest proteins. Osborne, in this country, had developed the methods of preparation of proteins and had demonstrated the individuality of a large number of them. Within the period of a few years Hopkins and Cole (90) discovered tryptophane, Fischer added proline, oxyproline, and valine to the list of amino-acids (55, 57) and showed that alanine, phenylalanine and serine were common constituents of proteins. Mörner (133) rendered the like service for cystine and Ehrlich discovered isoleucine (53). Beginning with the announcements of the polypeptide hypothesis the study of proteins became once more an occupation in which a self-respecting organic chemist might take a part. Within ten years many beautiful analyses of proteins had been made, the implications of the polypeptide hypothesis were thoroughly explored, many complex peptides were synthesized, and, most important of all, a foundation was laid for the study of the nutritive properties of proteins, which led directly to the discovery of vitamines and the complete revolution of the science of nutrition. The contrast between the first decade of the present century and the last decade of the previous century is remarkable. During the nineties fundamentally important chemical work on proteins was being prosecuted in only two laboratories, at Heidelberg and in New Haven. With the advent of Fischer into the field, protein chemistry attracted the widest attention and the results have been of the most far-reaching importance. If, as is stated by Mathews (123), Fischer was induced to turn his attention to protein chemistry by Kossel, the debt science owes this great biochemist is beyond estimation.

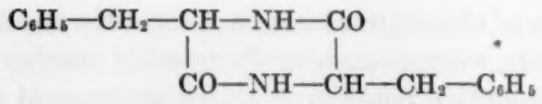
VI. Cyclic structure hypotheses. The polypeptide hypothesis of the constitution of proteins implies, on the simplest assumptions, the existence of a large and, probably, complexly branched chain of amino-acids. There was no inherent difficulty in this conception so long as the

molecular weights of the proteins were not regarded as excessively large. Fischer in 1917 (62), when discussing the possible number of isomers of a hypothetical polypeptide containing thirty amino-acid units and of a molecular weight of the order 3000 to 4000, stated his conviction that proteins of a molecular weight of the order 4000 to 5000 were not uncommon. This implies from thirty to forty amino-acids units. Inasmuch as straight chain polypeptides containing eighteen and nineteen units had been synthesized it was not difficult to imagine a stable chain twice as long.

As has already been pointed out, a body of evidence has recently accumulated which shows that egg albumin, although one of the simplest of the true proteins, has a molecular weight which is very close to 34,000 (44, 167, 174). This implies a peptide chain containing something like 250 amino-acids units. The molecule of hemoglobin is almost certainly twice as large (175) and that of the blood globulins larger still. We therefore have to do in many proteins, with peptide chains containing six and seven hundred units or even more. It seems highly probable that some configuration must be present which acts in such a way as to render the molecule far more compact than would be supposed upon the simplest and most obvious premises. It is useless here to speculate upon what this configuration may be, but the explanation of the difficulty will almost certainly be found when the nature of the bonds which are attacked by pepsin is finally discovered.

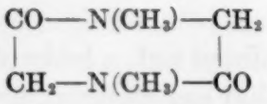
A number of attempts have been made in recent years to overcome some of the difficulties presented by the above considerations. Several of these attempts have been predicated upon a rejection of many views that have hitherto been regarded as well founded, and how such conceptions will fare remains to be seen. In any case it is obvious that it has become imperative to marshal our ascertained facts and to weigh carefully the evidence upon which a well wrought theory of the structure of proteins must be based.

A. *Abderhalden's diketopiperazine hypothesis.* Diketopiperazines have attracted attention since the early days of protein chemistry. The first member of the group, leucinimide, was obtained by Bopp in 1849 (33) and subsequently by Hesse and Limpricht (84) and by Kohler (102). Erlenmeyer and Lipp in 1883 (54) obtained the diketopiperazine of phenylalanine by dry distillation and suggested that formula III probably represented its structure.



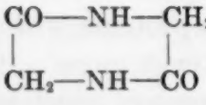
III. Phenylalanine diketopiperazine.

The following year Mylius (138) prepared the anhydride of sarcosine and, by means of oxidation to dimethyl oxamide and oxalic acid, showed that its structure was IV.



IV. Sarcosine anhydride

The constitution of the simplest member of the group, glycocoll anhydride, V, was established by Curtius and Schulz in 1890 (47).



V. Diketopiperazine (glycocoll anhydride)

These compounds received much attention from Fischer and his collaborators inasmuch as the first dipeptide, glycylglycine, was obtained by partial hydrolysis of the corresponding diketopiperazine (65) and subsequently many dipeptides were so prepared.

As already mentioned the possibility that the diketopiperazine ring might be a constituent of the protein molecule was pointed out by Fischer in 1906 (60), and this idea has been developed at great length in recent years by Abderhalden and his collaborators.

Abderhalden's idea, as expressed in one of his earlier papers on the subject (4), is somewhat revolutionary. It is suggested that the protein molecule is built up of a number of diketopiperazine-containing complexes which are associated or held together by means of secondary valences. The concept of a definite protein molecule is discarded; the "elementary complexes," to use Abderhalden's somewhat paradoxical phrase, may differ in number, constitution and arrangement in different proteins, but, since these are held together only by secondary valences, it is possible to conceive that, by changes in arrangement and perhaps the loss of a few of the complexes, alterations may take place that could even go so far as to convert one protein into another. The action of digestive enzymes upon proteins would consist, according to Abderhalden's view, first in the loosening of the secondary valences and the liberation of the

"elementary complexes," followed by the opening of the ring structure in these and ultimately, by the hydrolysis of the peptide bonds. Denaturation may be due to a change from the enol to the keto configuration in the anhydride ring, while the solubility of some proteins and the insolubility of others may be similarly explained. In short, many of the peculiar properties of proteins may be more or less plausibly accounted for in terms of this hypothesis.

The evidence upon which Abderhalden has based this view of the structure of proteins consists primarily in the observation that diketopiperazines can be isolated from the products of the partial hydrolysis of proteins (2). Numerous examples of the occurrence of substances of this type may be found in the literature. Thus Dakin isolated d-isoleucyl-d-valine from casein (48) and hydroxyprolyl-proline from gelatine (49). The great difficulty lies in proving that the ring structure so found does not owe its origin to the process through which the products of hydrolysis have passed. In the cases mentioned, the mono-amino-acid fraction had been heated with anhydrous butyl alcohol for many hours, an operation which might readily lead to condensation.

Experiments in which high temperatures have been employed are also open to question inasmuch as some polypeptides are known to condense under these conditions. Abderhalden and Komm (9, 10) have obtained diketopiperazines by heating various di- and tri- peptides with water, and also with dilute acid, at 150° and they strongly emphasize the possibility that anhydrides may be formed secondarily from polypeptides under certain conditions of hydrolysis.

Salaskin (158) obtained leucine anhydride from hemoglobin which had been digested 30 days with gastric juice. Unfortunately, however, he treated the material with alcohol at boiling temperature. Consequently if leucyl-leucine had been present there is a slight possibility that the trace of leucine anhydride he obtained may have been an artifact. The isolation of diketopiperazines from gelatine is of no significance as this material is prepared by heating connective tissue, etc., with steam under pressure.

In a few cases only have diketopiperazines been isolated from proteins by methods which render it probable that these existed preformed in the protein molecule. A substance obtained from edestin after pancreatic digestion was regarded by Abderhalden and Komm (14) as glycylproline anhydride, but the structure is unsupported by any evidence aside from a single analysis which fitted the theoretical requirements.

Perhaps the best supported preparation of a preformed diketopipera-

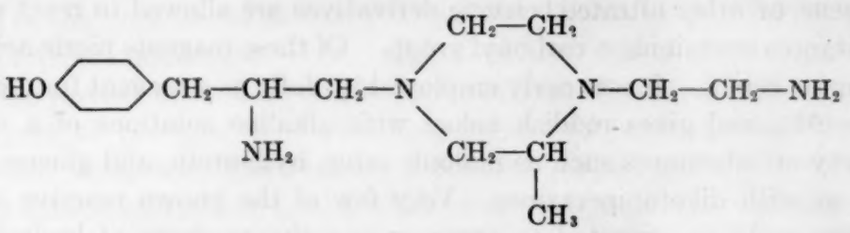
zine from protein was reported by Abderhalden in 1923 (3). Casein was boiled for two days with 5 per cent sulphuric acid and then heated at 80° with 10 per cent acid for four days more. After removal of the acid and concentration to dryness at low temperature the residue was pulverized and extracted with ethyl acetate. A crystalline substance was obtained from the extract (0.25 per cent yield) which was thoroughly identified as l-leucyl-d-valine anhydride. A compound which was probably d-alanyl-l-leucine was also isolated.

Abderhalden and Schwab (16) have also reported the isolation of an anhydride of alanine and serine from the products of the action of 70 per cent sulphuric acid at 37° upon silk fibroin. After removal of the reagent with great care to prevent rise in temperature, the fluid was evaporated and the residue extracted with ethyl acetate, whereby products which had the properties of glycyl-alanine anhydride and glycyl-tyrosine anhydride were secured. The residue was further extracted with hot alcohol which on cooling deposited a crystalline substance. On purification a product corresponding to synthetic alanyl-serine anhydride was obtained.

Abderhalden has also reported (5) the isolation of an anhydride of l-leucine and d-glutaminic acid from the products of pancreatic digestion of gliadin. The fluid was concentrated to dryness and the residue mixed with sand and extracted with a succession of solvents. The product was obtained from the methyl alcohol extract. It gave a positive reaction with picric acid (see below), and a negative reaction with ninhydrin. Unfortunately these properties would be equally well explained if the substance were a dipeptide of pyrolidone carboxylic acid and leucine. The evidence of synthesis was promised but has not yet appeared.

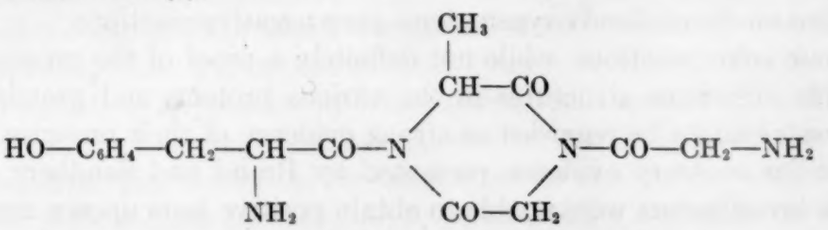
As another means of supporting his views of the constitution of proteins, Abderhalden and his collaborators (16) have attempted to isolate from partly hydrolyzed proteins substances which contain more than two amino-acid units, of which two are united in an anhydride ring. In order to stabilize any ring compounds present the so-called silk peptone, obtained by hydrolysis of silk fibroin with 70 per cent sulphuric acid for 3 days at 37°, was treated with sodium and alcohol. Abderhalden and Schwab were able to isolate methyl piperazine and 3-methyl-6-oxymethyl piperazine, obviously formed by the reduction of glycylalanine-, and alanyl-serine-anhydrides respectively from this mixture and, in addition, a substance was found which they regarded as a piperazine in which the four units, tyrosine, alanine, and two molecules of glycocoll were united. The methyl piperazine was readily obtained but the separation of the other constituents of the mixture was extremely diffi-

cult. No clear details of the preparation of the more complex substance are given and its constitution rests upon a single analysis of the hydrochloride that agrees only moderately well with the calculated requirements. The product, although white when freshly prepared, became yellow and finally brown on exposure to air and was somewhat hygroscopic. The constitution provisionally ascribed is given in formula VI, which indicates its relationship to the corresponding diketopiperazine.



VI. Tyrosyl-alanyl-glycine-piperazinyl-glycine

Abderhalden and Schwab also attempted the direct isolation of tyrosyl-alanyl-glycine-anhydride-glycine from silk peptone without previous reduction with sodium and alcohol. The hydrolysate was evaporated and the residue exhausted with ethyl alcohol and then extracted successively with methyl alcohol and chloroform. From both of these solvents a product was obtained by precipitation with ether which was purified by solution in dry methyl alcohol and precipitation with absolute ethyl alcohol. It formed a white crystalline powder giving a positive Millon's reaction even in the cold. The ninhydrin reaction was obtained slowly after continued boiling. On heating with alkali and neutralizing, the ninhydrin reaction became strongly positive. The picric acid reaction was positive, though faint. On complete hydrolysis tyrosine, alanine and glycocoll were obtained in amounts corresponding to 1, 1 and 2 molecules respectively and the analysis of the substance agreed satisfactorily with formula VII (4).



VII. Tyrosyl-alanyl-glycine-anhydride-glycine

The compound represented by this formula bears the closest relationship to the tetrapeptide glycyl-alanyl-glycyl-tyrosine, which Fischer and Abderhalden (64) isolated from silk fibroin.

It is unfortunate that Abderhalden and Schwab do not present more convincing and detailed evidence of the purity and constitution of this product since its significance for the substantiation of Abderhalden's views can hardly be overestimated.

Abderhalden and his collaborators have suggested, as further evidence of the presence of anhydride rings in the structure of the protein molecule, the color reactions that can be obtained when picric acid, m-dinitro benzene, or other nitrated benzene derivatives are allowed to react with substances containing a carbonyl group. Of these reagents picric acid is the most useful. It was early employed by Jaffe as a reagent for creatinine (94), and gives reddish colors with alkaline solutions of a wide variety of substances such as malonic ester, hydantoin, and glucose, as well as with diketopiperazines. Very few of the known reactive substances could be expected to occur among the products of hydrolysis of proteins and, of these, peptide anhydrides are the most probable. The reaction may therefore be employed as a diagnostic test for the presence of anhydride rings. Abderhalden and Komm (11, 12) have investigated the color reaction with picric acid, as well as other carbonyl group reagents, systematically. They found that no amino-acid or polypeptide gave the reaction while all diketopiperazines tested gave positive reactions with picric acid and sodium carbonate. Moreover the products of partial hydrolysis of proteins, the so-called peptones, which had been prepared with especial care to avoid possible synthesis of anhydride rings during the procedure, all gave positive reactions, as did several native proteins, particularly certain albuminoids. A few proteins such as edestin and legumin gave negative reactions with picric acid but positive reactions with m-dinitro benzene.

Various derivatives of diketopiperazines were also tested. Those which, from their method of synthesis, were regarded as N-N′ disubstituted substances gave positive reactions while those which were substituted on the enolized oxygen atoms gave negative reactions.

These color reactions, while not definitely a proof of the presence of peptide anhydride structures in the various proteins and protein hydrolysates might be regarded as strong evidence of their presence were it not for contrary evidence presented by Brand and Sandberg (34). These investigators were unable to obtain positive tests upon a number of proteins with either picric acid or m-dinitro benzene when they used a modification of the test which gave strongly positive reactions with diketopiperazines. They found that, on boiling with sodium carbonate and picric acid, arginine, tryptophane, histidine, and cystine gave weak

positive reactions although no test was given when these acids were boiled with carbonate, and aqueous picric acid added so as to form a supernatant layer. When casein, gelatine, and edestin were boiled with the reagents a positive test was obtained, but none when a sodium carbonate solution of the protein was boiled and treated with picric acid or m-dinitro benzene as already mentioned. The weak positive test obtained with these proteins on boiling may, therefore, conceivably be due to the presence of the above mentioned amino-acids rather than to diketopiperazine rings.

One further type of evidence has been submitted by Abderhalden in support of his view that diketopiperazines form constituents of the protein molecule. Abderhalden, Klarmann and Komm (8) have found that diketopiperazines, on oxidation with permanganate yield oxamide; the peptide glycyl-glycine likewise yields oxamide, but other peptides do not. Thus, with the one exception noted, the production of oxamide on oxidation may be considered as an indication of the presence of diketopiperazine rings in the material submitted to oxidation. Oxamide was obtained by the oxidation of the products of hydrolysis of silk prepared in the customary manner. The yield is not mentioned. Further studies by Abderhalden and Komm (13) indicated that oxamide could be obtained from partly hydrolyzed gelatine, casein and other proteins.

Although these experiments are suggestive they by no means prove that diketopiperazine rings are present in the protein molecule. The observation that glycyl-glycine yields oxamide on oxidation renders it necessary to obtain greater amounts of oxamide than can be accounted for by the amount of glycocoll in the protein before the evidence can be seriously considered. Moreover until more exhaustive studies have been made it cannot be certain that oxamide prepared by the oxidation of native protein may not have some other origin.

To sum up, Abderhalden's hypothesis that proteins are, essentially, complex compounds of which the components are substances containing amino-acids that are, in part at least, in peptide anhydride union with each other, is supported by experiments upon partially hydrolyzed proteins which show: *1*, diketopiperazines can be isolated from such material; *2*, piperazines can be isolated if the material is previously reduced with sodium and alcohol; *3*, positive color reactions for diketopiperazines can be obtained; *4*, oxamide can be obtained after oxidation of the material with permanganate.

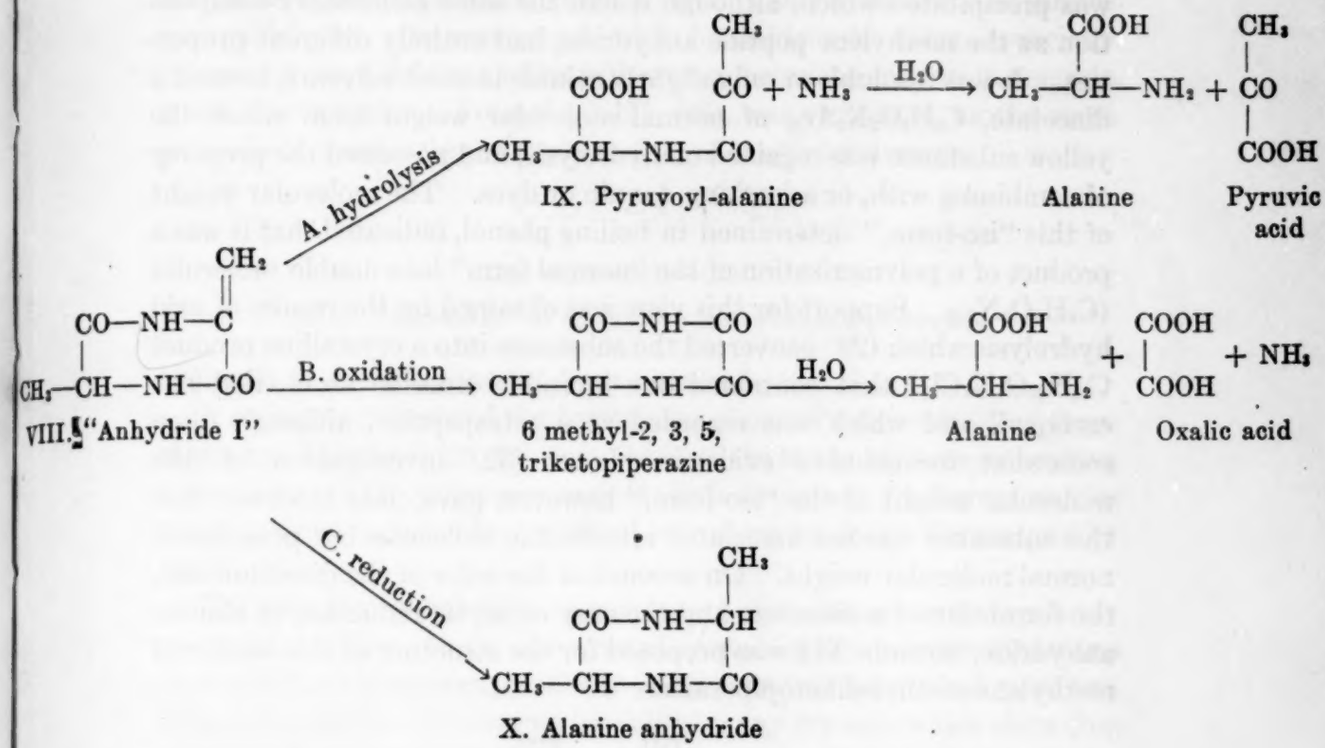
No one of these types of evidence is wholly conclusive, although it must be admitted that the sum total is impressive insofar as it presents a

strong probability that peptide anhydride rings are in some way involved in the constitution of the protein molecule. But in no case has any material proportion of the protein investigated been shown to be thus constituted and, moreover, few of the products have been identified in a wholly satisfactory manner, i.e., by ultimate analysis, hydrolysis, and isolation and identification of the constituent amino-acids, and lastly, synthesis. Although a compound of the type postulated as an "elementary complex" was isolated no evidence was presented that this substance could associate with others of a similar type to form compounds of high molecular weight. The chemistry of the diketopiperazines has been greatly extended by these investigations but it does not seem that the enormous labors of Abderhalden and his associates have really furthered his fundamental view of the structure of the protein molecule.

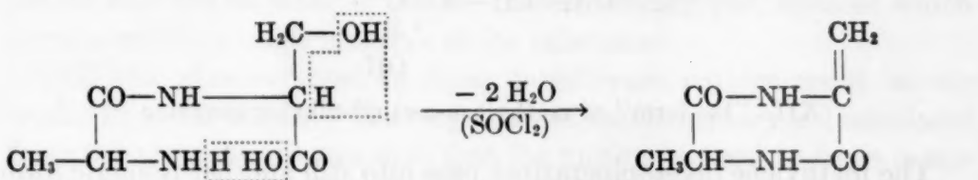
B. *Bergmann's hypothesis.* Although much is known of the chemical behavior of the simpler amino-acids glycocoll, alanine, and leucine and of their peptides and diketopiperazines, very little investigation has been made of the properties of peptides containing an amino-acid which possesses an active group in addition to the amino and carboxyl radicals. Bergmann and his collaborators have therefore begun the investigation of dipeptides and diketopiperazines which contain serine or cystine as one of their components. The behavior of alanyl-serine is, in many respects, typical of that of the other peptides they investigated (28, 30). This dipeptide, on treatment with thionyl chloride, yielded a chlorine-containing base, which, under the action of dilute aqueous ammonia, gave a relatively insoluble "anhydride I" $C_6H_8O_2N_2$. This substance, when dissolved in alkali and reprecipitated by neutralization with acid, was transformed into an apparently polymeric "anhydride II" to which the formula $(C_6H_8O_2N_2)_x$ was ascribed.

The structure of "anhydride I" was developed from the following reactions. (A) When heated with dilute acid one-half of the nitrogen was lost as ammonia, and pyruvic acid and alanine were formed, together with an acid which, on further hydrolysis, gave pyruvic acid, alanine and nitrogen-containing by-products. Since serine was not found among the hydrolysis products the pyruvic acid and ammonia must have had their origin in the serine part of the anhydride molecule. It is clear from this behavior that the third acid was pyruvoyl-alanine, IX. (B) The anhydride, on oxidation with ozone, gave a compound of high melting point and faintly acid properties which, on hydrolysis, gave alanine, oxalic acid and ammonia. (C) Reduction with hydrogen and palladium gave the well-known 3,6-dimethyl-2,5-diketopiperazine or alanine anhydride, X.

These three reactions are readily explained if "anhydride I" is assumed to be 3-methylene, 6-methyl-2,5-diketopiperazine, VIII. They may be formulated as follows:



The formation of "anhydride I" from alanyl serine under the action of thionyl chloride may therefore be represented by a reaction,

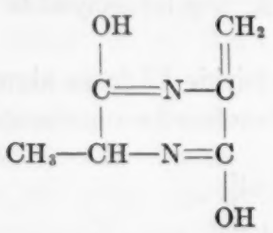


which differs from ordinary diketopiperazine formation only in the loss of the elements of a second molecule of water from the primary alcohol group and its neighboring carbon atom.

Glycyl serine yielded closely analogous products.

The most significant property of these new peptide anhydrides was

their behavior towards alkali. When dissolved in sodium hydroxide a yellow disodium compound was formed which could be crystallized. On neutralizing the alkaline solution a yellow substance ("anhydride II") was precipitated which, although it had the same elementary composition as the methylene peptide anhydride, had entirely different properties. It was insoluble or only slightly soluble in most solvents, formed a diacetate, $C_6H_6O_2N_2Ac_2$, of normal molecular weight from which the yellow substance was regained on hydrolysis, and possessed the property of combining with, or adsorbing, tannin or dyes. The molecular weight of this "iso-form," determined in boiling phenol, indicated that it was a product of a polymerization of the "normal form" into double molecules $(C_6H_8O_2N_2)_2$. Support for this view was obtained by the results of acid hydrolysis which (29) converted the substance into a crystalline product $C_{12}H_{22}O_6N_4Cl_2$,[9] that contained one ionizable chlorine atom, and one carboxyl, and which was regarded as a tetrapeptide, although upon somewhat inconclusive evidence. Later (32) investigation of the molecular weight of the "iso-form," however, gave clear evidence that this substance was not associated into double molecules but possessed a normal molecular weight. On account of the color of the disodium salt, the formation of a diacetate and the easy catalytic reduction to alanine anhydride, formula XII was proposed for the structure of this isomer of methylene-methyl-diketopiperazine.

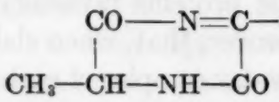


XII. "Iso-form" of methylene-methyl-diketopiperazine

The methylene diketopiperazines pass into still another isomeric form when they are heated with sodium acetate and acetic anhydride (27) or, more simply, on warming with a dilute aqueous solution of arginine, guanidine or ammonia in a sealed tube at 70–80° (31). The "allo" isomers so obtained are colorless when dissolved in dilute alkali, possess normal molecular weights in resorcin solution, do not yield crystalline sodium salts, do not form acetates and cannot be reduced by

[9] The exact formula of this compound is somewhat in doubt owing to misprints in the original article (p. 22, 23).

hydrogen and palladium. On acid hydrolysis, however, they yield so-called "tetrapeptides" very similar to, if not identical with, those obtained from the respective "iso-forms." The "allo" isomer of methylene-methyl-diketopiperazine absorbs tannins and dyes and also unites with aldehydes, the compound with formaldehyde being especially interesting since it forms jellies with water, behaving in certain respects much like gelatine. Formula XIII (26) was suggested as the most probable representation of the structure of the "allo" isomer,



XIII. "Allo-form" of methylene-methyl-diketopiperazine

although no proof has as yet been obtained.

In order to understand the hypothesis of protein structure that Bergmann has advanced (24, 25) as a result of the study of these interesting derivatives of the diketopiperazines, it is necessary to digress at this point in order to describe certain observations made by Herzog and by Brill.

The immensely valuable researches of the Braggs with the X-ray spectrometer have taught us to regard crystals as space-lattice structures in which the atoms occupy wholly definite and circumscribed positions. It is now possible to construct models of many crystals which show that the individual molecule loses its identity when the substance assumes the crystalline form, each single crystal being really a "super-molecule." Nevertheless it is possible to delimit a portion of the lattice model by planes that define what is termed the elementary cell, each of which corresponds to a single molecule of the substance.

Brill (36) observed that an X-ray interference pattern could be obtained from silk fibroin. The magnitude of the elementary cell calculated from the observations was such that the units appeared to have a molecular weight between the limits 500 and 660. By making certain assumptions, he reached the conclusion that the elementary substance consisted of four molecules of glycyl-alanine combined as an anhydride. Whether or not this conclusion is correct remains to be seen. The essential point is that the X-ray data appear to indicate the presence of regularly repeated units in silk fibroin of a molecular weight of the order 500–660.

Herzog and Kobel (83) have suggested other evidence supporting the view that proteins contain structure units of relatively small magnitude. They determined the molecular weight of silk fibroin by the depression

of the freezing-point using phenol as solvent, and reached the conclusion that this protein is dissociated in phenol into structure units that have molecular weights of the order of magnitude 200–400. Troensegaard and Schmidt (182) pursued the same method with gliadin and gelatin with analogous results.

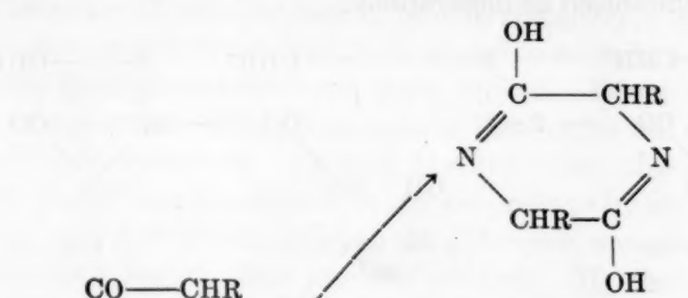
These somewhat startling observations have been explained by recent experiments of Cohn and Conant (42, 43), who were able to obtain similar results, although figures of a slightly higher order of magnitude for the molecular weight of the proteins investigated were found. They were struck by the fact, however, that, when elaborate precautions were taken to prepare thoroughly dry samples of material, even higher results were secured. They, therefore, added a drying agent, anhydrous calcium chloride, to the phenol and, after determining the freezing point of the system, phenol-anhydrous calcium chloride-hydrated calcium chloride, introduced a carefully dried sample of protein. After the final traces of water still adhering to the protein had been absorbed by the drying agent, the system showed no detectable change in the freezing point. In other words, the depressions of the freezing point previously observed by themselves, and probably also by Herzog and others, were due to traces of moisture adhering to the preparations subjected to the test. The method, consequently is useless as a means of ascertaining molecular weights of proteins and all conclusions drawn from such determinations are invalid.

Bergmann accepted the results of Brill and the molecular weight determinations of Herzog and suggested that the relatively small structure units of which proteins appear to be composed, are held together by forces which differ only in magnitude from those acting upon the units in the space-lattice of a crystal. They are analogous to the forces acting in Werner's "coördination compounds" (26) and are valences of a secondary type. He believes that the most promising method of arriving at a knowledge of the nature of the protein structure units is by a study of synthetic model substances and, while he is not prepared to assert that the isomeric methylene diketopiperazines are actually involved in the constitution of any protein, he is of the opinion that many of their properties fulfill the chemical and physical requirements of a protein structure unit. Thus hydrolysis of the "iso" or "allo" modification yields products which are apparently of peptide nature but, inasmuch as these products are derived from substances which are certainly of cyclic constitution, it is clear that the formation of peptides by the hydrolysis of proteins is not necessarily evidence that these are preformed in the protein molecule. Furthermore, since it is apparently impossible to

obtain the fundamental "normal" methylene diketopiperazine from the isomeric "iso" and "allo" forms, it is evident that there is no necessity for the structure unit which lies at the basis of the protein molecule to turn up when the protein is decomposed by chemical reagents or by enzymes.

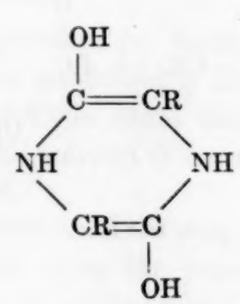
These ideas of Bergmann, which have many features in common with those of Abderhalden, were expressed at a time when the "iso" and "allo" forms of the methylene diketopiperazines were thought to be substances of high molecular weight, a view based in part upon their capacity to adsorb tannin and dyes. The chief objection to them is the lack of any trustworthy evidence that proteins are in reality composed of aggregates of small molecular weight held together by secondary valences. Further there is no experimental evidence that the methylene diketopiperazines or their isomers are attacked by enzymes. On the other hand the preparation of new types of compounds derived wholly from amino-acids is of great importance to protein chemistry and Bergmann has made a valuable contribution to the fundamental chemistry of amino-acids.

C. *Karrer's synthetic compounds.* The simple formulation of the diketopiperazine ring (XIV) which is usually given by no means exhausts the possibilities of variation in the structure of these substances. Enolization of the keto groups may take place in two directions leading to compounds of the structures XV and XVI.



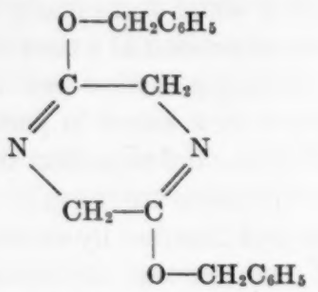
XIV. Diketopiperazine

XV. Pyrazine



XVI. Enolized diketopiperazine

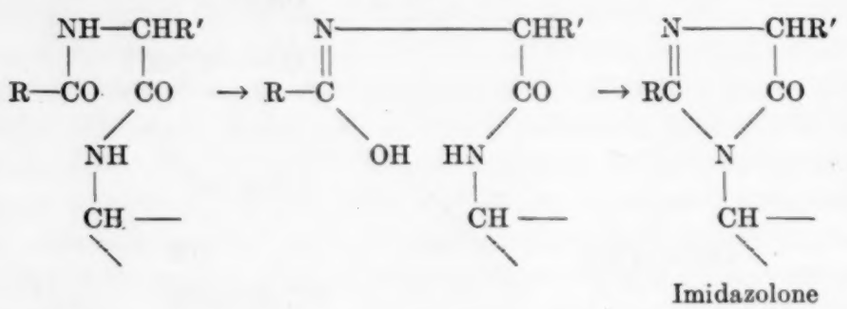
Karrer and his collaborators (96, 97) have prepared O-O′ disubstituted compounds of diketopiperazines which they formulate according to the first scheme, that is, as pyrazine derivatives. Such a substance (XVII) was obtained by the action of benzyl chloride upon the silver compound of glycine anhydride.



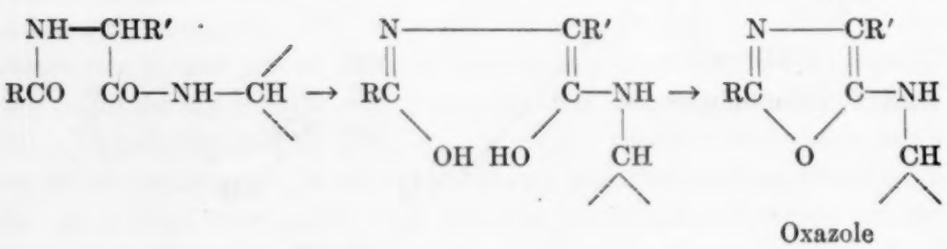
XVII. O-O′ dibenzyl diketopiperazine

The interest in these compounds lies in the fact that they are extremely sensitive to the action of dilute acids, being readily hydrolyzed with the formation of glycocoll and, in this case, benzyl chloride. Formulae of analogous ring structures may be written which, on hydrolysis, would yield nothing but amino-acids and oxy-amino-acids.

Still further possibilities of labile ring structures may be imagined as a result of enolization of polypeptides. A number are given by Karrer of which two are reproduced as illustrations.

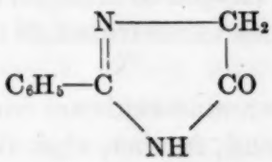


Imidazolone



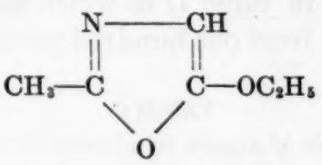
Oxazole

Compounds of these types as well as others have been obtained. Thus hippuric acid on treatment with phosphorus pentachloride gave the imidazolone XVIII,



XVIII. 2-phenyl imidazolone.

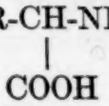
while acylated amino-acids gave compounds of the second type, for example 2-methyl-5-ethoxyoxazole (XIX) from acetyl glycine ester (98).



XIX. 2-methyl-5-ethoxyoxazole

These ring structures are also very easily decomposed by acids, and similar compounds can, theoretically at least, be constructed entirely of amino-acids. Karrer therefore draws attention to the fact that these model substances are of some interest in protein chemistry. As yet, however, no compounds constructed entirely of amino-acids have been prepared and the effects of enzymes upon them studied. When this is done the relative importance of ring structures of these types will become evident.

D. *Ureide hypotheses.* Lippich in 1914 (120) observed that more carbon dioxide was liberated from certain proteins by alkaline hydrolysis than by acid hydrolysis and that the difference was greater than could be accounted for by their arginine content. He therefore suggested that uramino-acids, R-CH-NH-$CONH_2$, might occur in the protein



molecule. This idea was further emphasized by Andersen and Roed-Müller (18), who pointed out that a considerable body of evidence favored this view. This, however, is little more than a suggestion which attempts to account for the small amount of carbon dioxide that is set free by the hydrolysis of proteins.

Brigl and Held (35) have critically examined the current speculations of Abderhalden, Bergmann and Karrer upon the constitution of pro-

teins, have dismissed them and, following the principle of building upon and developing older and better tested views, have suggested a modification of the polypeptide hypothesis of protein constitution. They point out that the ratio of oxygen to nitrogen atoms calculated from the ultimate analyses of proteins varies from 1.28:1 in lactalbumin to 1.12:1 in elastin (see table C).

If it is assumed that the amino-acids are combined in the protein as a single huge polypeptide, and, further, that the ammonia nitrogen obtained by hydrolysis has its origin in amide groups, it is possible to calculate a theoretical ratio of oxygen to nitrogen atoms from the available amino-acid analyses, provided that the analyses account for a sufficiently large part of the protein molecule. Such a calculation, taken from Brigl's paper, is shown in table D in which columns 1 and 2 give the yield of the amino-acids from one hundred parts of lactalbumin, column

TABLE C

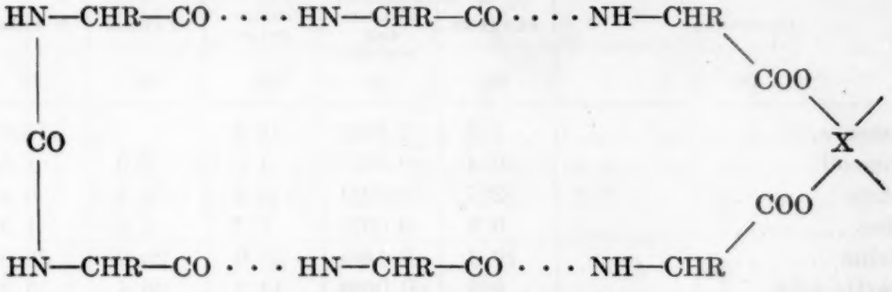
The ratio of oxygen to nitrogen in proteins

	OXYGEN	NITROGEN	$\frac{\text{O ATOMS}}{\text{N ATOMS}}$
	per cent	*per cent*	
Lactalbumin	23.13	15.77	1.28
Serum albumin	22.31	15.89	1.23
Casein	22.73	15.65	1.27
Fibroin	26.0	19.2	1.18
Elastin	21.44	16.76	1.12
Zein	20.78	16.13	1.13

3 is obtained by dividing each figure in column 2 by the the molecular weight of each amino-acid, and column 4 is obtained by dividing each number in column 3 by the common factor 0.0053. The last two columns give the number of oxygen and nitrogen atoms to be ascribed to each amino-acid molecule provided all are in peptide union in the protein. It is necessary to note, however, that the dibasic acids occur partly as amides and partly with free carboxyl groups. The number of molecules of the former type will correspond, presumably, with the number of ammonia molecules. A dibasic acid molecule with a free carboxyl group will have three oxygen atoms per molecule while one with an amide group will have two. Oxyglutaminic acid will have four atoms of oxygen. In Brigl's table the amide groups are arbitrarily assigned to all of the aspartic acid and to three molecules of glutaminic acid.

The ratio between oxygen and nitrogen atoms, as thus calculated,

comes out at 1.15:1 which is sufficiently far from the ratio 1.28:1 required by ultimate analysis (Table C) as to demand explanation. Brigl and Held reject the view that low results for the determination of oxy-amino-acids can be entirely responsible for the larger amount of oxygen that is obviously present in lactalbumin and believe that oxygen in some other form must be present. They suggest that proteins consist of chains of polypeptides which are joined to each other by the end-standing groups through links which contain oxygen. The most probable linkage for the amino groups is by means of a ureide group while the carboxyl radical may be regarded as linked by ester groups upon polyvalent alcohols (XX).



XX. Brigl's structure hypothesis

It is essential that both ends of the chain should be thus combined as, otherwise, the protein would be a predominantly acid or, on the other hand, basic substance.

The hypothesis agrees with the results of enzyme studies which indicate that the protein is first split into a number of large polypeptide fragments. Experiments upon synthetic substances constructed according to this model have not yet, however, shown that such ureide bridges can be split by enzymes (see also (15)).

Unfortunately for this hypothesis an examination of the data for the analysis of zein, which is by far the most completely analyzed protein (94.4 per cent actually assigned, table E, column 3, as against 68.4 per cent for lactalbumin, table D, column 3), shows that there is no such need for the postulation of oxygen-containing groups as Brigl and Held suppose. In table E are given results that have been compiled from the best existing analyses of zein. The figures in the last four columns have the same significance as those in table D. The oxygen atoms in the dibasic acids have been totalled and diminished by 69, the number of nitrogen atoms which may be assumed to be in amide combination and which are therefore substituted for an equal number of oxygen

atoms. The ratio of 341.6 to 318.2 is 1.074 while, as is seen from Table C, the ratio of oxygen atoms to nitrogen atoms in zein is 1.13. Thus in the only protein that has been adequately analyzed the excess of oxygen atoms is by no means striking. Further, by assuming somewhat higher figures for the oxy-amino-acids serine and oxyglutaminic acid, the ratios may be brought into even closer agreement. If it is supposed that each of these is present to the extent of four per cent the figures in parentheses in the table show the necessary changes and

TABLE D

Lactalbumin

AMINO-ACID (1)	PER CENT (2)	PER CENT MOLECULAR WEIGHT (3)	MOLECULES (4)	O ATOMS (5)	N ATOMS (6)
Ammonia	1.5	0.0882	16.6		16.6
Glycocoll	0.4	0.0053	1.0	1.0	1.0
Alanine	2.5	0.0281	5.3	5.3	5.3
Valine	0.9	0.0077	1.5	1.5	1.5
Leucine	19.4	0.1481	28.0	28.0	28.0
Aspartic acid	9.3	0.0699	13.2	26.4	13.2
Glutaminic acid	10.1	0.0687	13.0	36.0	13.0
Cystine	1.7	0.0141	2.7	2.7	2.7
Serine	1.8	0.0171	3.2	6.4	3.2
Oxyglutaminic acid	10.0	0.0614	11.6	46.4	11.6
Arginine	3.5	0.0201	3.8	3.8	15.2
Lysine	10.0	0.0685	12.9	12.9	25.8
Histidine	2.7	0.0174	3.3	3.3	9.9
Proline	4.0	0.0348	6.6	6.6	6.6
Phenylalanine	2.4	0.0145	2.7	2.7	2.7
Tyrosine	0.9	0.0050	1.0	2.0	1.0
Tryptophane	3.1	0.0152	3.0	3.0	6.0
	84.2	0.6841	129.4	188.0	163.3

those at the foot give the relative numbers of oxygen and nitrogen atoms that would then be present in this protein. Their ratio is 1.127, in complete agreement with the requirements of ultimate analysis. It is therefore clear that in this protein at least no unusual source of oxygen need be sought.

A further objection to Brigl's view is the wholly indefinite nature of the groups which are supposed to bind the carboxyl radicals of the polypeptide chains. No suggestion is made other than that they may be

TABLE E

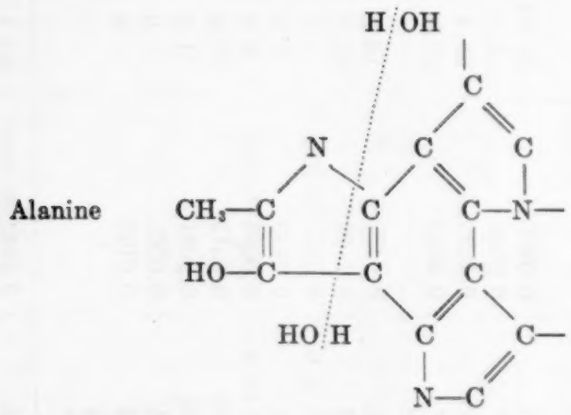
Zein

AMINO-ACID (1)	PER CENT (2)	PER CENT MOLECULAR WEIGHT (3)	MOLECULES (4)	O ATOMS (5)		N ATOMS (6)
Ammonia	3.64*	0.2141	69.06	0		69.06
Glycocoll	0.0*					
Alanine	9.8†	0.0981	31.64	31.64		31.64
Valine	1.9*	0.0162	5.2	5.2		5.2
Leucines	25.0†	0.1905	61.4	61.4		61.4
Proline	9.0*	0.0782	25.2	25.2		25.2
Oxyproline	0.0†					
Phenylalanine	7.6†	0.0461	14.9	14.9		14.9
Glutaminic acid	31.3†	0.2129	68.7	206.1		68.7
Oxyglutaminic acid	2.5† (4.0)	0.0153 (0.0245)	4.9 (7.9)	19.6 (30.6)	169.6 (181.6)	4.9 (7.9)
Aspartic acid	1.8†	0.0135	4.3	12.9		4.3
Serine	1.0* (4.0)	0.0095 (0.0381)	3.0 (12.3)	6.0 (24.6)		3.0 (12.3)
Tyrosine	5.66‡	0.0313	10.9	21.8		10.9
Cystine	0.75‡	0.0031	1.0	1.0		1.0
Histidine	0.8§	0.0051	1.6	1.6		4.8
Arginine	1.8§	0.0103	3.3	3.3		13.2
Lysine	0.0§					
Tryptophane	0.0*					
	102.56	0.9442 (0.9810)	304.1	341.6 (372.2)		318.2 (330.5)

* Osborne and Liddle (149).
† Dakin (50).
‡ Looney (121).
§ Kossel and Kutscher (107).

polyvalent alcohols. Such compounds or their decomposition products have not been found among the products of hydrolysis of simple proteins.

E. *Troensegaard's pyrrole hypothesis.* In 1920 a paper was published by Troensegaard (178), which contained a wholly new speculation upon the constitution of proteins. He was impressed by the fact that the pyrrole ring plays an important part in current views of the constitution of chlorophyll and of hemin, and that pyrrolidine derivatives are found among the products of hydrolysis of proteins. Moreover substances giving the pine splinter reaction, supposed to be characteristic for pyrrole derivatives, are found among the products of the dry distillation of proteins. He therefore believed that the pyrrole ring may be a far more important constituent of the protein than has hitherto been supposed and has suggested a skeleton model (XXI) which is constructed of amino-acid residues so disposed as to form pyrrole rings condensed with each other.



XXI. Troensegaard's structure hypothesis

He pointed out that if the rings were decomposed on hydrolysis as indicated by the dotted line, they would yield the usual amino-acids; consequently, if such structures were to be demonstrated in the protein molecule some other type of decomposition must be employed. He therefore acetylated the proteins he investigated (gliadin and gelatin) under anhydrous conditions and then, in order to stabilize the rings, reduced the product with sodium and amyl alcohol. The subsequent steps were designed to separate the various basic and acidic fractions thus obtained. The fundamental test applied for the presence of pyrroles was the pine splinter reaction. In later papers (179, 180, 181) these views were further developed with vast experimental detail.

Troensegaard's ideas have attracted a great deal of attention, undoubtedly far more than they merit. His papers, nevertheless, contain a valuable suggestion. The method of decomposition he employed leads to products of a new type and it will be necessary to reconcile these products with current structure theory. Moreover, he pointed out that investigators have hitherto pursued a single method of decomposition in their studies of proteins and that such a procedure may well have led to a somewhat one-sided view of the molecular structure.

There are a number of fatal objections to the formula suggested by Troensegaard as a model of the constitution of proteins. In the first place there is no evidence that enzymes attack such structures. Secondly, the hydrolysis of the double bonds that are indicated should lead to optically inactive amino-acids whereas protein amino-acids are all, with the exception of glycocoll, active. Thirdly, the central structure in this formula is the benzene ring which is one of the most stable configurations known. It is improbable that this ring would split up to yield amino-acids in the manner suggested by Troensegaard. It is not sufficient to urge that phenylalanine and tyrosine do contain the benzene ring and are obtained from proteins. The ring indicated in this formula is substituted with three carbon atoms. Finally no definitely characterized pyrrole or pyrrolidine derivatives have been separated from proteins in a pure form by Troensegaard's methods.

F. *The enzyme studies of Waldschmidt-Leitz.* As has been already mentioned the final test of a structure which is assumed to form part of the protein molecule is the behavior of synthetic model substances towards the enzymes which attack proteins. Enzymes are among the most specific and delicate reagents with which chemists are familiar and a demonstration that a particular configuration is attacked by them is excellent evidence that such a configuration plays some part in the natural substrate of the enzyme. The converse of this statement is not necessarily true, however, since it is an essential feature of enzyme activity that all structural peculiarities must be reproduced in the model. Consequently a negative result with enzymes does not completely preclude the possibility that the model may have certain configurations in common with the natural substrate. Thus the wholly negative results that have hitherto been obtained when diketopiperazine derivatives (6, 7, 188) or ureide derivatives (35) are exposed to the action of proteolytic enzymes do not demonstrate that such configurations may not occur in the protein although they do raise a reasonable doubt.

Willstätter and Waldschmidt-Leitz and their collaborators have,

in recent years, devoted much attention to the preparation of the proteolytic enzymes in as pure and uniform a condition as possible (187). Their results promise to shed light upon the constitution of proteins and have already helped to clarify the present views of the nature of enzyme action. The two proteolytic enzymes of the pancreas and of the intestines, trypsin and erepsin, were successfully separated and preparations obtained that were regarded as homogeneous at least as respects the enzyme content. They distinguish four enzyme types, pepsin, inactivated trypsin, activated trypsin and erepsin and have arrived at conclusions somewhat at variance with the older views of Fischer and Abderhalden (63), who distinguished between peptides which are attacked by trypsin and erepsin and those which are not. Waldschmidt-Leitz has found that all the tested dipeptides containing natural isomers of the amino-acids were split by erepsin from whatever source while none were attacked by trypsin whether this was activated or not. On the other hand erepsin had no effect upon native proteins (casein, gliadin, etc.) nor upon artificial peptones or protamine although all of these were attacked by activated trypsin. Unactivated trypsin, however, attacked only peptone and the protamines. Thus the activating material, enterokinase, is regarded as a necessary adjunct to the digestion of native protein.

The action of the four types of enzyme appears to be fairly clearly defined although a certain amount of overlapping could be detected (189). Thus peptic action could still be observed upon a substrate obtained by the action of tryspin on casein; but when trypsin and erepsin had completed their action on casein no substrate remained upon which the action of pepsin could be detected. Waldschmidt-Leitz is of the opinion that the primary action of pepsin is to cleave the protein molecule into a number of large fragments, which, since they are readily attacked by erepsin, are most probably polypeptides for erepsin is the one enzyme which is known to hydrolyze the peptide bond. On the other hand it is equally logical to assume that the bonds which are not attacked by erepsin but which are decomposed by the other enzymes may be of other types. Although their exact nature is still unknown (185) it is significant that, when broken, they give rise to carboxyl and amino groups in approximately equivalent proportion.

These experiments emphasize the importance of the peptide bond in protein structure although they show that bonds of other types are likewise present. The discrepancies between these results and the older experiments are readily understood when it is recalled that in their

original work on synthetic peptides, Fischer and Abderhalden employed enzyme preparations which must have been far from homogeneous. It is clear that Waldschmidt-Leitz and his collaborators have provided investigators with tools which will inevitably play an important part in future attempts to comprehend the details of the structure of proteins.

CONCLUSIONS

The cyclic structure hypotheses for the most part emphasize the possibility that amino-acids can enter into combination with each other in ways that were not envisaged at the time the peptide hypothesis was proposed. The fundamental chemistry of amino-acids has thus been greatly extended. The difficulty has been, and still is, to perceive a connection between the synthetic compounds of these new types and the protein. Hypotheses that disregard well-established facts of protein chemistry do not tend to promote progress, and it is clear from the above discussion that the cyclic structure hypotheses are not in accord with the experimental results of Sörensen, of Svedberg and of Cohn upon the molecular weights of proteins; nor do they, with the possible exception of Bergmann's hypothesis, pay adequate attention to the results of the analytical studies begun by Fischer and Kossel and continued by Abderhalden, Osborne, Dakin and Jones, which emphasize the enormously complex constitution of the protein molecule. In short the peptide hypothesis of Hofmeister is still, in spite of certain shortcomings, the foundation stone of protein chemistry. Developments there must be, but it is not the method of science to proceed by revolution. Striking advances in chemical thought, with few exceptions, have had their roots firmly fixed in the past. The future development of protein structure theory may be most confidently looked for in the direction of an expansion of the peptide hypothesis.

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THE SIGNIFICANCE OF THE T WAVE IN THE ELECTROGRAM AND ELECTROCARDIOGRAM

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The significance of the T wave has been the subject of debate almost from the time that its presence was recognized in the action current of the heart. Many disputed issues have been eliminated as the methods of registration have improved; of the issues that remain, some are based on opposite viewpoints, but many are merely aspects of the same phenomenon viewed from different angles, and therefore may be looked upon as supplementing one another. This will become clearer as the various phases of the subject are reviewed.

1. *History.* The presence of a variation in the action current of the heart equivalent to the T wave was known before the advent of the Einthoven string galvanometer. It was first recognized by Köllicker and Müller in 1856, and its presence was confirmed by Meisner and Cohn in 1862 (cf. A4, A5, A7), who noted that a twitch of the rheoscopic frog preparation occurred occasionally at the onset of diastole, as well as at the beginning of systole. Donders in 1872 (cf. A1) recorded these twitches graphically and worked out their time relation to the myocardiogram; he is thus to be regarded as the first to make a permanent record of the action current of the heart.

A new method of registration, more direct than the preceding, was soon available in the form of various types of reflecting galvanometers and rheotomes developed by Matteucci, du Bois-Reymond, Hermann and Bernstein for physiological use (cf. D6). Curves from the heart were obtained with this type of apparatus by Englemann in 1873 (cf. A1), and soon after by Marchand in 1877 (A6), and by Burdon-Sanderson and Page in 1880 (G13). The curves recorded in this way were diphasic. Those obtained by Burdon-Sanderson and Page showed in addition a distinct isopotential period between the two phases.

A further refinement in technique was afforded by the introduction of the capillary electrometer by Lippmann in 1872 (cf. D6). The action current of the frog's ventricle was first recorded with this instrument by

Marey in 1876 (cf. A5). In his curves and in the more extensive series published by Burdon-Sanderson and Page in 1883 (G13), the initial and final phases were distinct. A similar type of record was obtained from the isolated mammalian heart by Waller and Reid in 1887 (K76), by Bayliss and Starling in 1892 (K10), and by von Kries in 1895 (G24), the animals employed being dogs and cats. The first electrocardiogram of man was made by Waller in 1889 (K74) and soon thereafter human electrocardiograms were published by Bayliss and Starling, 1892 (K10), by de Vogel, 1895 (cf. A2) and by Einthoven, 1895 (A2). Einthoven corrected his records by Burch's method. These corrected records show a close resemblance to the records obtained with the string galvanometer which he later introduced (A3).

2. *The action current of the heart in various species.* The action current of the heart of a large variety of animal species has been recorded either by direct or indirect leads. The records published or described include those obtained from many species of cold-blooded vertebrates (fish, amphibians and reptiles) besides the commonly employed laboratory frog and turtle (B1, B4, B8, B11, B21, B26, I 14, K1, K2, K3, K5, K21), from birds (B3, B5, B14, B15, B16, B20, B22, B23, B25, K5), from species of mammals besides the dog and cat (B2, B7, B9, B10, B13, B17, B18, B19, B24), and even from invertebrates of the mollusc and crustacean orders (B6, B11, B12, K3, K5, K21). All of these records show a characteristic initial and final deflection except some obtained by Hoffmann (B11) from certain species of Limulus and some by Evans (B6) from the snail. Tigerstedt (K5) attributed the oscillations which replace the final phase in Hoffmann's records to the moribund condition of the animals employed. The double peaked or adult character was also noted by several authors (B5, B20, B22, B25, K5) in the curves obtained from the chick embryo. According to Wertheim-Salomonson (B25), this contour first appears at the time that the heart muscle assumes its characteristic striated appearance; but the opinion has been questioned (H5).

Records have been obtained not only from the whole animal, intact heart and isolated ventricles, but also from isolated chambers of the mammalian ventricles (K27), from the isolated auricle of cold-blooded animals (B1, C2, C3a, C4, G22, I 22, K5, K21, K69), from the vena cava of the frog (K21), from the bulbus arteriosus of the frog (K21), from strips of turtle's and frog's ventricle (C5, G18, G18a, K19), and from strips of mammalian auricle (C3, C5a), in all of which a final (or T) deflection distinct from the initial (or QRS) was observed. Further-

more, a separation of the auricular complex into initial and final deflections has been noted in the human electrocardiogram (C6) and in that of other vertebrates (B18, C1, C5b). The presence of a final deflection in all these species indicates identical sources of origin.

THE SOURCES OF TISSUE ELECTRICITY. The discussion of the T wave, as of any galvanometric deflection obtained from living tissues, naturally centers about those changes which produce variations in electrical potential. The manner in which these deflections are recorded precludes the idea that they are due primarily to changes in electrical resistance (D1), although resistance changes probably enter into their formation secondarily. All the possible sources of tissue electricity are fundamentally attributable to orientation of ions (D10) or of electrically polarized molecules (D69). It is unlikely that frictional electricity plays any part (D1). In spite of the large amount of work done on this subject, the exact mechanisms by which the potentials are created is still unsettled. Only the more pertinent facts and prevailing opinions can be considered in the present review. For further details, the reader is referred to the original articles on this subject included in the bibliography, and to the excellent summaries given by Bayliss (D1), Bernstein (D2), Beutner (D3), Davis (D5), Fulton (D6), Höber (D7), Lillie (D8, D9) and Michaelis (D10).

1. *The resting polarized state.* It is now generally conceded that resting living tissues are electrically polarized. The evidence for this opinion is fairly complete. Some of the facts supporting it are: 1. The effect of the cathode and anode of the galvanic current on living cells is the same as on artificial polarization cells (D26, D27, D46a). 2. There is a difference in the resistance to the passage of the electric current in the two diameters of the nerve—a difference which disappears at death (Hermann referred to by Ebbecke, D27). 3. The resistance offered by tissues to the passage of a constant current is greater than to the passage of an alternating current (D7). 4. The potential of the current of injury changes only when the temperature of the uninjured region is varied and not when that of the injured area is altered (D11, D16). 5. A potential difference appears when two uninjured regions of resting nerve or muscle of different temperatures are connected to a galvanometer (D67, D73, D75). 6. The potentials of the current of injury of the individual muscles summate when connected in series, i.e., injured to uninjured surface (D20). 7. The effect of different electrolytes and different concentrations of the same electrolyte on the potential of living cells is the same as on the potential of polyphasic chains or

chains with interposed semipermeable membranes (D7, D17, D25, D41, D50, D54, D67, D74).

It is now generally believed that the polarization or orientation of electricity occurs at phase boundaries. This belief results from the weight of evidence accumulated since the time of Ostwald (D59), the first proponent of this conception. It is now recognized for example that surface energy, adsorption, and, possibly, the chief chemical energy exchanges of an oxidative-reductive nature (D76) all occur only at phase boundaries. Attention has been directed mainly to the surface of the cell—which is generally held to be also semipermeable because certain electrolytes cannot enter the resting living cell (D55a, D66),[1] and because all cells while alive have a characteristic composition differing from their environment both as to amount of colloids and crystalloids (D1, D30, D44). Phase boundaries, however, occur within the cell, and may be concerned with the polarization of resting cells.

The evidence seems to establish the fact that the semipermeable layer at the surface is polarized, though how such a condition is produced is not yet clear. Whether this layer is simply an adsorbed one or a distinct phase of more than dimolecular dimensions is still controversial (cf. D2). Some authorities have considered the surface as a distinct precipitation (D60) or colloidal membrane (D25, D41, D51, D74), semipermeable in nature, which gives rise to a Helmholz double layer by virtue of a Donnan equilibrium (D1, D7, D13, D47, D53, D71) or by protracted differences in the diffusion rates of ions of unlike charge through the membrane (D18). Others have considered the surface layer as a distinct lipoid (D3, D17, D22, D37, D58) or non-aqueous phase (D57, D57a) producing a prolonged potential by virtue of the unequal partition coefficient of ions of opposite sign in the two phases. There is still no agreement as to the nature of the ions actually involved in either of the above mechanisms (D7, D16, D24, D32, D40). The view has also been advanced that the polarized state is not caused by the presence of semipermeable membranes, but by an orientation of electrically polarized molecules at the cell surface (D69, D70); an orientation that might be produced by surface forces. Such oriented molecules have been invoked to explain not only the action current and current of injury, but also excitation, conduction (D69) and muscular contraction (D33). Still another view attributes the polarized layer directly to oxidation-reduction potentials (D42) (it must be assumed at the same time of course that the processes of oxidation and

[1] See also D1, D8, D40, D44a.

reduction occur at opposite sides of the cell membrane). The positive wave of the auricle (E1, E2, E3, E4, E5) and nerve (F1, F2, F3, F4, F5) (the presence of the former is still controversial (E1, E2, E5)), the effect of temperature referred to above, and the difference in potential between tissues of different resting metabolism rates (D42) could be readily explained as variations in oxidation-reduction potential across the surface membrane. The mere fact that the temperature coefficient of the current of injury is low (D2) does not rule out such oxidation-reduction potentials, for if a physical process, with a lower temperature coefficient, is associated with the chemical, the coefficient of the whole process will be low (D1). It is apparent that further investigation is still needed to determine the mechanisms by which a polarized state in resting tissues is produced.

2. *The changes during activity.* The commonly accepted theory for the action current and current of injury assumes that stimulation and injury lead to an increased permeability and depolarization of the cell surface, resulting in a potential difference between the polarized and depolarized regions (D2, D8, D9, D14, D15, D46, K4). It is further assumed that tissue activity tends to restore the resting state of polarization and semipermeability of the surface (D5, D9, G1, G2). This theory has also been used to explain the phenomena of excitation and conduction (D2, D8, D9, D33a, D39, D45, D46, D48, D55, G1, G2), and within the last few years it has been applied to similar phenomena in heart tissue by Schellong (D63, D64, D65).

The idea of preëxisting polarization in tissues, first suggested by du Bois-Reymond, was denied by many of the earlier writers such as Hermann (cf. D4) and Oker-Blom (D56). The latter authors claimed that the action current and current of injury arise de novo at the site of excitation or injury because of the difference in velocity with which the newly formed ions of unlike charge diffuse away from the site of their formation. Brünings (D20, D21) showed, however, that taking into consideration the amount of acid products formed, the potential developed in this fashion was a hundred times smaller than the potential actually observed. The fleeting nature of such potentials in the body (D1), and the fact that temperature changes at the injured area have no effect on the potential (D16) would also rule out this possibility.

There is considerable evidence to show that during activity a depolarization and an increased permeability actually occur. Of this evidence the chief is the demonstration of: 1, an increase in permeability to certain substances during activity (D23, D29, D43, D52); 2, a decreased

resistance to the passage of an electrical current during activity (D2, D49, D61, D68); 3, a concomitant increase in permeability and electrical conductivity in plant cells on passing an electric current through them (D12, D28, D77); and 4, changes in metallic models similar to those in nerves following stimulation (D8, D9, D18a, D19, D72). The fact that both the pole of the constant current which decreases polarization of the cell and the chemical substances which increase its permeability act as stimulants (D8, D9, D26), also favors this view.

The increase in oxygen consumption (D31, D35) and the two-phase heat production (D34) accompanying nerve excitation are definite proof that chemical changes accompany excitation. The causal and temporal relationship between the chemical, physico-chemical, and electrical changes which are associated with excitation, is, however, as yet not definitely established (D8, D9, D36, D38, D62). If we attribute the normal polarized state of tissue to an oxidation-reduction potential, we must assume that this potential is reduced or reversed during excitation; if we attribute it to oriented electrically polarized molecules, then their orientation must be altered during excitation; if we attribute it to the semipermeable nature of the surface membrane, then this is altered during excitation. The action current can be explained in any one of these ways, but which, if any, actually takes place is still a matter of speculation. All we can assert with certainty is that the cell is depolarized and its surface layer made more permeable during activity, and that the re-creation of the normal polarized state and original semipermeable nature of the cell surface is accomplished soon after.

THE REASON FOR THE APPEARANCE OF THE T WAVE IN THE ELECTROGRAM.[2] 1. *The monophasic action current.* Any attempt to explain the T wave in the electrogram by describing the mechanism by which changes in tissue potential might arise is futile unless cognizance is taken of the duration, magnitude and contour of the potential change so produced in the neighborhood of each of the two electrodes (I 16). It is because this fact has not always been clearly appreciated that a great deal of polemic has arisen in this field.

Attempts were made to determine the duration, magnitude and con-

[2] The genesis of the T wave in the curve recorded with leads directly on the heart, or on strips or portions of it, is not the same as that of the T wave recorded with indirect leads (I 19, J6). Samojloff (I 19), therefore, introduced the terms electrogram and electrocardiogram to distinguish between records taken with direct and those obtained with indirect leads. In this review Samojloff's terminology will be followed.

tour of the action current in different regions of the heart by some of the early investigators, e.g., Burdon-Sanderson and Page (G13), Bayliss and Starling (K10), and von Kries (G24), and since then by many others (G1, G2, G5, G6, G7, G11, G12, G15, G18, G18a, G21, G22, G27, G28, G29, G30, G31, G32, G33, G34). The method employed by these investigators consisted simply in destroying the vitality of the region around one electrode either by chemical, mechanical or thermal means (G10, G19, G29). The deflection recorded under these conditions, the so-called monophasic action current, would presumably measure the potential change under the electrode on the uninjured area, provided, 1, the method gave a quantitative measure of actual potential differences, 2, the electrodes measured only the potential in their immediate neighborhood, and 3, no uninjured tissue remained, or was restored, in the injured area. It has been recognized, within recent years, that not one of these assumptions is absolutely true.

The circuit through the galvanometer is not the only one through which the electric current can flow between the two electrodes; other circuits are provided by the fluid layer which surrounds the tissue between the two electrodes and by the intercellular fluid layers (D48, G1, G2, G4, G5, G14, H6). The deflection of the galvanometer, depending as it does on the intensity (amperage) of current flowing through it, will measure in addition to the potential difference between the two electrodes, the variable and as yet undetermined relative resistance of the tissue shunts (H6). Even the employment of electrometers, oscillographs, etc., does not remove this source of error (G4, G5, G18a).

The galvanometer records, in addition to the potential changes occurring immediately beneath the electrodes, those of a variable region around them (G1, G2, G5, G15, G24, G25) depending on the proximity and size of the electrodes (G1, G2, J6), on the sensitivity of the galvanometer employed (G9, G14, G17), and on the amount of extraneous conducting fluid present around the tissues (G4, G5, G14, I4). Thus, Drury and Brow (G15) were able to get a deflection even after the areas beneath their electrodes were destroyed, and Lewis, Meakins and White (G25) obtained a deflection after blocking the passage of the impulse to the region of the auricle on which the electrodes were placed. (See addendum 1, p. 500.) These so-called extrinsic effects (G25) tends to prolong and distort the deflection. The contour and duration of the action current beneath the electrode on the uninjured area can therefore only be approximated. The best approximation of the action current in any region is obtained when small electrodes

lying close together are used (G2). The use of inactive tissue near the active tissue, for a leading off point, for example tendon, is not equivalent to the use of an injured area, for the tendon or other inactive tissue acts merely as a prolongation of the electrode (G3, G5).

The inability to destroy completely the deeper layers of muscle beneath the electrode, and also the tendency of all living tissue to restore the polarized state of its exposed surfaces (G23), a tendency particularly rapid in the mammalian heart, both lead to further distortions of the curve (G1, G2, G4, G5, G8, G29, G33).

Smooth monophasic action currents can be recorded on leading off from injured-uninjured areas provided the injured area is extensive, and provided the records are taken immediately after the injury is made (G2, G29, G33). These smooth curves consist of a steep ascent, followed by a prolonged horizontal or slightly declining summit, which terminates in a fairly rapid descent. The descent is always more protracted and gradual than the ascent. While such smooth curves are regarded by many as the true form of the potential changes in an uninjured cardiac region (G1, G11, G24, G29, G33, L2), they are not always easy to obtain. Certain variations frequently appear. They consist usually of: 1, a downwardly directed small deflection, of small amplitude and short duration, preceding the main deflection, and 2, the replacement of the single protracted summit by a double summit—the first, brief in duration and spike-like in contour, and the second, protracted in duration and arch-shaped. In such double peaked curves the dip between the two summits remains considerably above the zero potential line.

The small preliminary dip has been ascribed to the earlier appearance of activity in the uninjured tissue lying near the injured area, which therefore has a greater effect on the lead on the injured than that on the uninjured area (G29, G33). The double summit, on the other hand, has been attributed to the presence of activity near the electrode on the injured area, which appears late and lasts a short time because of its proximity to the injured area (G29, G33). The presence of the double summit may also be due to the fact that the action currents of the various fibres are out of phase, a fact demonstrated to be true in nerve (G16, G26). This last source of distortion can be avoided by recording the action current at the site of stimulation as Gilson (G18, G18a) and Bishop and Gilson (G5) have recently done.

These deviations of the monophasic curve have been supposed to be the actual changes under the electrode on the uninjured area. Jolly

(G22) expressed the opinion that the dip preceding the main deflection actually indicates that a positive variation precedes the negative in active tissue. The presence of a double summit has been used to support the view that two distinct processes contribute to cause the action current, namely, excitation and contraction (H1, H4, H6, K39, L26, L27), or fibrillary and sarcoplasmic activity (G6, G27, G30, H2, H3, I2, K1). The probability of such a dual origin is considerably reduced in view of the plausibility of the alternative explanation for the appearance of a double summit, given above (G29, G31, G32, I23, I24). In fact, advocates of the dual origin hypothesis will find it difficult to explain why a smooth monophasic curve can be recorded immediately after the injury is produced, and why it gradually becomes changed to a double summited curve, and finally to a typical electrogram, as the time between the production of the injury and the taking of the record is increased. This has been found to be the case (G29, G32), and has been readily explained as a distortion due to the increase in the amount of active tissue in the region of the electrode on the injured area, as recovery from the injury proceeds (G29, G32).

2. *The electrogram as the resultant of the interference of two monophasic action currents.* The prevalent explanation for the contour of the electrogram, the so-called interference theory, considers the electrogram as the algebraic summation of the monophasic action currents recorded by each individual electrode (G7, G8, G13, G18a, G21, G22, G24, G28, G29, G32, G33, G34, I1, I16, I24). Several advocates of the idea that the source of the action current is double have also concurred in this point of view (G27, I2). Mechanical devices have been constructed to show how activity in two regions interfere with each other (I15, I20).

According to some of the proponents of the interference theory, the two monophasic action currents of opposite sign are considered to have the same contour, amplitude, and duration, but to be out of phase because of differences in the time of their onset (I2). According to this conception, if the two monophasic action currents were in phase they would neutralize each other and no deflections would be recorded; on the other hand, the size of the deflections would become larger as the difference in time of onset of the individual monophasic action currents increased. If this simple conception were true, the contour of the T wave would bear a definite relationship to the initial deflection (cf. I2). While such a relationship frequently does occur, it is, at least, just as frequently absent (G33, I3, I5, I8, I10, I12, I16, I17, I18, I19, I19a, I21, I22, I23, K10, L37). It has been assumed, to explain the independent

variation of the initial (QRS) and final (T) deflections of the electrogram, that there are differences of duration and contour in the monophasic action currents of different regions, in addition to the differences occurring in the time of their onset (G29). This has been inferred from the observation that the duration of the monophasic action current is readily changed when the conditions of part or of the entire heart are modified (G7, G8, G12, G13, G18a, G20, G28, G33); and has been actually observed to occur in different regions of the heart of the cold-blooded animal (G7, G8, G20, G24, G27, G34). Yoshida (G33) has shown clearly that the theoretical electrogram calculated from the actual monophasic action currents recorded by the two electrodes agrees very closely with the actual electrogram observed. His study was made under a variety of conditions, where the duration of the individual monophasic action currents varied considerably. Briefly, his technique consisted in applying three electrodes to the frog's ventricle, one at the apex, one at the base, and the third on an injured area in the middle, and then recording in quick succession the deflection given by connecting apex with middle, middle with base, and apex with base. The first two curves recorded the monophasic action currents beneath the basal and apical electrodes from which he calculated the theoretical electrogram, and the last curve recorded the actual electrogram.

Variations of the T wave independent of the initial deflection have also been obtained with the differential electrode by Garten and his pupils (I4, I6, I8). This fact has been taken to indicate that the duration of the action current is unequal even in adjacent regions (K41).

The argument, that the independent variability of the T wave is proof that its origin is due to a type of tissue activity different from that causing the initial complex, is of little value in view of the preceding evidence which indicates that the deflections of the electrogram are caused by interference of potential changes of similar origin but occurring out of phase in the two regions (cf. I24). This fact has been admitted by at least two of the advocates of the so-called dual origin theory (G27, I2).

The theory advanced by Gotch (I9) and by Kraus and Nicolai (I11), which has been supported by Waller (K75) and recently by Freud (I7), namely, that the T wave in the electrogram obtained on leading off from base and apex is due to the return of excitation to the bulbar region of the heart, is no longer tenable (I10, I14, I23, I24) in view of the presence of such a T wave in the differential electrogram (I4, I6, I8), in isolated portions of the heart (see p. 448), and even when the bulbar region is

removed (I14), or blocked off from the rest of the heart (K72). Furthermore this theory of conduction does not agree with the pathway of invasion mapped out by Lewis (I13) for cold-blooded animals, and by Lewis and Rothschild (K48)—confirmed by Wiggers (K79)—for warm-blooded animals.

The evidence, at present available, supports the hypothesis that, in direct leads, the character of the T wave is determined by the algebraic summation of the last portion of the monophasic action current recorded by each of the electrodes. The monophasic action currents of different regions of the heart are not in phase because of differences in time of onset; and, in addition, are different in contour and duration.

THE REASON FOR THE APPEARANCE OF THE T WAVE IN THE ELECTROCARDIOGRAM. 1. *The relation of the electrogram to the electrocardiogram.* The chief difference between the electrogram and the electrocardiogram lies in the relation of the electrodes to the source of the heart's currents (I13, I19, J1, J5, J6, J8). In direct leads, the regions in the neighborhood of the electrodes have a much greater influence on the galvanometer than more distant ones, especially when an extirpated empty heart is employed (J1). In the electrocardiogram this disparity in influence of various regions is practically absent. Consequently, we deal not only with the potential changes in individual regions but even more with their spread and retreat (J6). Just as the electrogram is to be considered as the algebraic resultant of the monophasic action currents recorded by each of the two electrodes, so the electrocardiogram is to be regarded as the first differential quotient of the electrical stresses occurring in the heart from moment to moment oriented in the direction of the lead (K41). The cause for the deflections in the two types of leads is not necessarily alike.

2. *The electrical field of the heart.* It has been the opinion of many workers, including the pioneer Waller (K74, K76), that the heart in situ in the body is equivalent, during activity, to an electrical battery placed in a relatively large mass of conducting medium—an electrical battery which varies in potential and in the position of its poles during the heart cycle (J6, J7, K3, K4, K6, K19, K26, K29, K31). In fact, Craib (K19) has recently shown that the electrical field of the heart obeys with reasonable accuracy, during the time of the initial as well as that of the final phase of the action current, the mathematical formula of such a type of battery, or as he prefers to call it an electrical doublet or bipole. Lewis (I13, K4) has used this conception in mapping out the direction and intensity of the electrical current in each ventricle from moment to

moment during the early part of the period of invasion. And it is on this basis that the three standard leads of the electrocardiogram were established (J2).

Any analysis of the electrocardiogram, to be complete, must include not only an evaluation of the manner in which the electrical field of the heart is produced and its properties varied, but also an estimate of the influence of the extra-cardiac tissues and of the position of the electrodes on the contour of the action current (J6). The part played by the location of the electrodes has been recognized for some time (J2, K29, K31), and actually worked out in artificial models by Clement (I4) and Schellong (J6). The latter, in particular, has shown that the intensity of the current flowing through the galvanometer is determined, as regards both its intensity and direction, by the angle between the electrical axis of the artificial electrical doublet and the line connecting the leads. He found further that the distances between the electrodes and their relative distance from the artificial source of current also had a great influence on the intensity and direction of the current flowing through the galvanometer. The effect of the electrical resistance offered by the extra-cardiac tissues, especially that caused by polarization and capacity changes of the skin, is also important, as Einthoven and Bijtel (J3) have shown. The opinion has been expressed that changes in extra-cardiac resistance and skin capacity in ascites, edema and myxedema, may explain the changes in the amplitude of the electrocardiographic deflections found in these conditions (J4, J7, N67).

There is as yet no unanimity of opinion as to the manner in which the heart in the body produces its electrical field during its activity. There are two main points of view, which Lewis (K4, K47) has called, respectively, the theory of distributed potential differences, and the theory of limited potential differences. According to the former theory, the reason for the appearance of an electrical field around the heart is the fact that activity (or excitation) does not start or cease simultaneously in all regions (J8, K6, K9, K10, K23, K24, K29, K30, K31, K38, K55, K77); and the further fact that in certain phases of the cycle activity starts simultaneously in non-adjacent regions, because of the branching of the Purkinje system (K6, K30, K31). The same facts hold true also during the retreat of excitation (K6, K30, K31). Currents of electricity flow between the active and inactive tissue regions because a difference in electrical potential is established. The flow of current outside the heart, which we tap with the galvanometer, is the algebraic resultant of of the currents set up from moment to moment in the various regions of

the heart (K31). The effect can be compared to that produced by a group of electrical batteries irregularly placed in a conducting medium. Carrying this analogy further the negative pole of the heart is located on the side of the electrical equator of the employed lead where the greatest amount of active tissue (unit negative poles) is present at the moment, and the potential which is measured is determined by the relative amounts of active (unit negative poles) and inactive tissue (unit positive poles) on the two sides of this equator at that moment (K6, K30, K31). This view is in reality an amplification of the interference theory of the nature of the electrogram to fit the conditions present in indirect leads.[3]

Certain facts have recently led Lewis (K47), Einthoven (K25), and Craib (K19) to question the validity of the foregoing hypothesis and to postulate the theory of limited potential differences. Lewis' objection to the older theory deals only with the period of invasion (K47). He points out that the direction of the components of the initial phase of the action current in the amphibian heart does not coincide with the distribution of the regions involved from moment to moment, but rather with the direction in which the excitation is spreading. He found, also, that stimulation of closely adjoining epicardial and endocardial surfaces of the right ventricle of the dog caused the first part of the initial complex to be oppositely directed in the electrocardiogram obtained with electrodes applied to the right and left side of the chest wall. This would not have been the case, he claimed, if the location of the fibres were alone responsible. He assumed, to explain his results, that the initial phase of the electrocardiogram was the algebraic resultant of the currents set up by the electrical doublets which were produced between adjacent active and inactive tissue as the excitation wave advanced. The presence of a small positive deflection preceding the main negative one, in leading off directly from injured-uninjured areas, which Jolly (G22) considered as showing that a positive deflection preceded the negative in each region, might be additional support for this view of limited potential differences. There is however another explanation for the presence of this occasional dip (see p. 454). Perhaps the most conclusive evidence for the theory of limited potential differences was recently given by Craib (K19). This investigator found that the polyphasic character of the initial deflection of a strip of tortoise ventricle placed in a large mass of conducting medium did not agree with the conception of distributed potential differences, but did agree remarkably well with the idea that the onset of excitation was accompanied by an electrical

[3] Craib's criticism of this view will be considered later (cf. p. 461).

doublet of short duration; a similar result was obtained in the case of the mammalian heart. (Gilson (G18a), however, was unable to confirm Craib's observations.) On the whole, the evidence for such a doublet at the onset of excitation seems complete. Whether this doublet is located between adjacent active and inactive tissues as required by Lewis' theory (K47) or Eiger's hypothesis (K21, K22), or between the two ends of the individual electrical unit, presumably the fibre, as Einthoven's (K25) and Craib's (K19) theories require, is still unsettled. Lewis does not attempt to explain how the doublet is produced. Eiger attributed it to a difference in the diffusion velocities of the newly created positive and negative ions. Such a doublet would be of short duration, would have its positive pole in the inactive tissue, and would advance with the excitation wave. Both Einthoven and Craib expressed the belief that the doublet arose in each electrical unit for a short time as the unit was excited.

While Lewis was cautious not to assume a similar limited potential difference during the retreat of excitation, Eiger, Einthoven and Craib did apply their respective theories to the period of retreat. Eiger assumed that an orientation of ions occurred during recovery from excitation, the doublets produced at this time following the same path taken during the invasion, but being reversed in sign. The more gradual and protracted character of the recovery process, which tends to avoid the great localized differences in composition capable of causing momentary diffusion potentials during excitation, makes it unlikely that an orientation of ions occurs during the retreat of excitation. Craib, without defining the cause, assumed that in each electrical unit of the heart the brief doublet appearing during the invasion was followed by a prolonged doublet of opposite polarity, which gradually waxed and waned. No conclusive proof has been brought forward as is demanded by Craib's hypothesis—and Eiger's as well—to show that a positive phase does follow the main negative one in the monophasic action curve obtained when leading off directly from injured-uninjured areas. There is no need to invoke Craib's hypothesis to explain the occurrence of the T wave. All the changes in the T wave are more readily explained, and with much fewer assumptions, by the older view of distributed potential differences, as will be shown presently (cf. p. 463). A similar criticism can be raised against Einthoven's hypothesis that the T wave is dependent on the time relation of the recovery process in the ends of the fibre, rather than on its regional distribution.

De Boer (K14, K15) has recently brought forward experimental

evidence which apparently is contradictory to the idea that local electrical doublets are responsible for the direction of the major deflections of the electrocardiogram. This evidence was obtained from the frog's ventricle. In his experiments he divided the ventricle into an upper and a lower half, leaving a narrow muscular bridge between, which slowed conduction and served to delay the passage of the impulse between the upper and lower half. The preparation was allowed to beat in a natural manner and the deflections recorded by leads applied directly to auricle and apex as well as by indirect leads in the same plane. The deflection obtained during the excitation of the upper half of the ventricle was in the opposite direction to that recorded later when the lower half was excited. This could not have been the case if the direction of spread or retreat were responsible for the deflection, but only if the distribution of active and inactive tissue in relation to the electrical equator of the lead had been the cause, for de Boer maintained that the general direction of spread and retreat was the same in the two halves.

To sum up: It has yet to be shown that limited potential differences, in the sense used by Lewis and Craib and implied by Eiger, enter into the formation of the final (T) phase of the electrocardiogram though the evidence available at present makes it seem that limited potential differences are the sole cause or, more probably, combine with distributed potential differences in causing the initial (QRS) phase. There is no need to postulate that the T wave is caused by limited potential differences in view of the adequacy of the hypothesis of distributed potential differences to explain the facts.

The criticism which Craib (K19) has made against the hypothesis of distributed potential differences, namely, that there is no reason for assuming that the remaining region of the heart acts as a positive pole when the active region acts as the negative pole, is very easily answered. Obviously the proponents of the hypothesis of distributed potential differences do not refer to an absolute but rather to a relative positivity, implying thereby that the "zero" level of the whole heart, as of the whole body, has shifted. In absolute terms this shift is toward the potential present in the active tissue.[4] If we consider the heart a syncytium—and in the present state of our knowledge this view is just as reasonable as the alternative view that the heart is composed of individual discrete cells—then it follows that as soon as a region becomes active it will tap the inside of the rest of the heart's syncytium. The

[4] Such a change in potential has been noted by Voegtlin, de Eds and Rosenthal (Jour. Pharm. Exper. Therap., 1928, xxxiii, 259).

active region will therefore act as the negative pole, and the rest of the heart, which is inactive, will of necessity, act as the positive pole, the source and sink of the electrical potential being ultimately referable to the two sides of the intact membrane or surface (cf. p. 450). This state of events will continue until the polarized condition is restored in the active region. Certain regions gain an advantage because the intensity of current flow varies not only with the difference in potential, but also with the amount of electrical resistance between regions having such differences of potential. Obviously, the greatest intensity of current flow will be between adjacent active and inactive regions, as the resistance is least here (cf. J6). For this reason the adjacent active and inactive regions will appear to act as the two poles of the source of the bioelectric current, although in reality it is the two sides of the intact surface layer or membrane in the inactive region which is the source (cf. D8, D9, J6).

3. *The cause of the appearance of the T wave in the electrocardiogram.* Perhaps the greatest stumbling block in accounting for the T wave has been the fact that in the electrocardiogram of mammals this wave is usually in the same direction as the chief of the initial deflections. Any explanation, to be adequate, must account for this fact. It must also explain why changes in the T wave can occur independently of the changes in the initial deflection. Such variations have been noted as a result of the action of epinephrin (A5, K41, K69, K78, L4, N22), morphine (K28), digitalis bodies (K12, K13, K17, K18, K50, K54, K56, K59, K60, K65, K69, K84, N66), insulin (K36, K44, K51, K61, K83) and other drugs and chemicals (K2, K5, K8, K16, K28, K32, K33, K37, K41, K42a, K62, K63, K64, K70, K78, K84, N76); they are also noticeable as a result of local changes in temperature (I8, I21, K10, K34, K67, K80, K82), or of nutrition (K11, K27, K40, K68, N31); and have been observed in general alterations of the conditions of the animal (K42, K46), or of the circulatory system (K41, K45, K57, L4). (See addendum 2, p. 500.)

The apparent independence of the initial and final deflections in the electrocardiogram has led some investigators to believe that the two deflections are of different origin, as in the electrogram[5] (B25, G30, H1, H3, H4, K1, K27, K37, K39, K43, L27); and others to believe that the excitation process returns again to the base (I9, I 11, K75). This last opinion is no longer tenable as we have seen (p. 456) (see also K48, K52, K53, and K72). The criticism of the idea that the source of the T wave

[5] See also H3a.

is different from that of the QRS deflection is the same as that already raised when discussing the electrogram (see p. 456).

It is not necessary to postulate either of these theories to explain the independence of the initial and final deflections. It is much simpler to assume that the duration of excitation is not the same throughout the heart, and that consequently the pathway of retreat is not the same as the pathway of invasion (I8, K9, K10, K23, K24, K30, K31, K41). Such an unequal duration of the action current in various regions of the heart as has already been mentioned, is indicated directly by the duration of the records obtained on leading off directly from injured-uninjured areas (see p. 456); and indirectly by the fact that the manifest potential during the inscribing of the T wave is not opposite in direction to that obtained at the peak of the R wave (K30).

Many investigators have ascribed the normal upright T wave to a longer duration of excitation in the base of the heart (I8, K9, K10, K23, K24, K29, K35, K71), in the right ventricle (K23, K24, K39, K40, K49, K55, L48), or, what is more likely, to the fact that excitation does not cease latest in the region which is excited latest (K30, K31, K38, K41, K77). The fact that a T wave which is variable in its relation to the initial deflection occurs in the differential electrogram (I8), indicates that the duration of excitation is not the same even in closely adjacent regions. It would seem, therefore, that the apparent persistence of excitation in any area is really caused by the unequal duration and non-simultaneous onset of excitation in many adjacent fractions of the heart which leads to the unbalancing of stresses during the retreat of excitation (K41).

There can be no doubt that the time of excitation of the various regions plays a definite rôle in determining the time of offset, especially when conduction to a large area is considerably delayed (K4, K81, K82). But it is also true that differences in the duration of excitation in the various regions play at least as important a rôle (K41, K80). This is true even in bundle branch block as is indicated by the lack of relationship occasionally noted between the initial and final deflections during the onset of branch block (K58).

A great many investigators, led by the discovery of the His-Tawara system in the ventricles and the practical value of considering the initial phase as a biogram, have considered the T wave of the electrocardiogram in the same way (K7, K49, K65, K66, K81, K82). It is apparent, however, that we cannot actually record the last part of the levo- or dextrogram by cutting a bundle branch, for the last part of the

curve so obtained will still be a combination of the stresses of both ventricles, in which the time relations of excitation in the two chambers are markedly altered (K40). The only way of obtaining the contour of the entire levo- or dextrogram would be to block completely the passage of the impulse into one or another of the ventricles. The attempts carried out (K27) have not been entirely successful because of the large amount of trauma involved.

But even if we did know the contour of the whole levo- and dextrogram, we would still have to explain the appearance of a final deflection in terms of a summation of activity of the individual fractions of the ventricle. Such a subdivision can therefore serve no useful purpose unless it be shown that the T wave bears some relation to the relative strength of contraction of the two ventricles as has been maintained by Daly (K20), Hoffmann (K39), Kraus (K43), Usoff (K73) and others. It is difficult to see how the relative strength of contraction can be mirrored in the electrocardiogram unless we accept Usoff's opinion (K73) that it is due to a twisting of the heart's anatomical axis during contraction, in which case it is equivalent to a shifting of the position of the electrodes during this time. De Meyer's (H4) hypothesis resembles Usoff's in this respect. In both, some other cause for the appearance of an electrical field around the heart is implied. They explain only how this field might be distorted. There can be no question that such a shifting of the heart as Usoff implies does occur, but how far it makes the T wave indicative of the relative strength of the contraction of the two ventricles is open to question. It is interesting to note that Gilson (G18a) has shown that the change in potential produced by stretching the heart, the so-called de Meyer phenomenon, is only about 5 per cent of the potential of the T wave.

To sum up: The evidence at present available indicates that the T wave in the electrocardiogram is caused by the unbalancing of electrical stresses during the retreat of excitation which are in part the result of a non-simultaneous onset of excitation, and in part the result of an unequal duration of excitation in the various fractions of the heart.

THE RELATION OF THE T WAVE TO THE OTHER CHANGES OCCURRING DURING THE CARDIAC CYCLE. 1. *The relation between electrical and mechanical events.* The entire subject of the quantitative and temporal relationship of the action current to the other changes which accompany the activity of the ventricles is at present controversial. Recently, even the apparent difference in the time relation between electrical and mechanical manifestations of activity in cardiac and skeletal muscle

has been questioned. As ordinarily recorded the durations of the electrical and mechanical records are roughly the same in cardiac muscle, while in skeletal muscle they are markedly different (cf. D6, G2, G12, G21, G29, I24, L3, L10, L25, L38, L51). This difference has been used by some as proof that in the electrocardiogram there is added to the electric manifestation of excitation, a manifestation of contraction, the T wave. "T waves" have, however, been recorded in the electromyogram under normal conditions or during fatigue (G5, L9, L30, L39, L40). Fulton (D6) maintained that these secondary waves, which he was also able to get, were artefacts due to shifting of the electrodes. Bishop (L9) and Bishop and Gilson (G5) have apparently eliminated this source of error and still found "T waves." These latter authors showed that the action current records obtained on leading off from injured-uninjured areas in skeletal muscle were more prolonged than generally believed. In fact, they insisted that the short monophasic action currents previously recorded were due to the obliteration of the slowly declining descent by interference from the active tissue in the neighborhood of the injured region.

For a long time it seemed clear that electrical variations could occur even when contraction was absent. Many investigators have recorded more or less typical electrograms and electrocardiograms after shortening and the development of tension had entirely disappeared from the heart (I16, I21, K38, L14, L24, L37, L41, L44, L46, L67), and as a matter of fact, Klewitz (L37) found that the effect of local temperature changes on the T wave was practically the same whether or not there was any evidence of contracton. These observations have been cited to refute the idea that contraction is responsible for the T wave (I16, I21). Within recent years, however, Arbeiter (L2) Einthoven and Hugenholz (L15), and Joachimoglu and Mosler (L28) maintained that the disappearance of the action current and mechanical evidence of contraction went hand in hand. Similarly, Fulton (L19), contrary to Beritoff (L8) and von Brücke (L11), found a parallelism in the disappearance of the electrical and mechanical evidence of activity in the skeletal muscle. Einthoven and his co-workers (L1, L2, L15) pointed out justly that the methods previously employed to record mechanical changes were not comparable in sensitivity with those employed to record electrical changes. They maintained that the failure of previous observers to find a parallelism in the disappearance of the electrical and mechanical manifestations of activity showed the inability of the recording devices to determine small amounts of shortening or tension

changes. They perfected a much more sensitive instrument to record the mechanical changes.

The Einthoven school has gone a step further. De Jongh (L29) claimed, and he was upheld by Einthoven (L1), that the onset of shortening and of the action current were simultaneous to within a few thousandths of a second. While it is true that the durations of the mechanical and electrical manifestations of activity are about the same in the heart (G7, G8, G12, G20, G21, G29, I16, I24, L54), and vary in the same direction as a result of various procedures (G20, I10, L6, L10, L44), the majority of investigators have failed to find this simultaneousness of onset (A1, H1, H4, K1, L5, L16, L18a, L20, L21, L27, L31, L44, L47, L50, L52, L57, L60, L64, L65, L66). This does not necessarily refute de Jongh's observation, for it is probable that the difference observed by most investigators is due only to the relatively insensitive instruments employed to record the mechanical events. However, further evidence apparently against the idea that mechanical and electrical changes start simultaneously is given by Kleinknecht (L36) in the heart, and by Beritoff (L8), Fisher (L18) and von Brücke (L11) in skeletal muscle. These investigators observed a considerable variation in the time relation of the onset of the action current to mechanical change when the experimental conditions were modified. Similar variations were noted by Garten (L20) and Wiggers (L64). The entire subject merits further investigation with more sensitive methods of recording the mechanical changes.

The time relation of the T wave to mechanical systole is variable; consequently, the durations of mechanical and electrical systole are not the same (L4, L52, L64), nor are the changes the same quantitatively when conditions are altered (K41, L4, L12, L17, L52). In fact, the changes are sometimes in the opposite direction (K41, L4, L52). The value of such comparisons of duration and time relation varies with the accuracy of the method used to register the mechanical activity of the ventricles. The employment of the apex beat as a time measure of mechanical activity (L42, L63) for such comparisons is of doubtful value because of the variability in its contour (K8). Similarly, the use of the x-ray shadow (L7, L23) is also of little value (L53). Perhaps, the best evidence of the time values of mechanical activity, at present available for the intact heart,—though not free from error—is obtained from accurately recorded heart sounds and pressure pulses. The relation of the T wave to the second heart sound has been found to be quite variable (K2, K5, L5, L12, L13, L16, L22, L27, L31, L33, L49, L61,

L62, L64, L66). Sands (L52) and Bartos and Burstein (L4) have shown that this relationship changes in the same animal as conditions are altered. Similar evidence has been obtained on comparing the T wave with the carotid pulse (K24, L34, L63), aortic pressure curve (K41), and left ventricular pressure curve (K41, L20, L32, L48, L64). The presence of an asynchronism in the cessation of the mechanical systole of the two ventricles, suspected by von Zwaluwenburg and Agnew (L69) and demonstrated by Katz (L35), might lead to the suspicion that a better relationship existed between the action current and right ventricular activity. This is not the case, as is shown when the comparison is made with the right auricular (L21), venous (K73), pulmonary arterial (K41), and right ventricular pressure curves (K41, L32).

The variability between the T wave and the end of right and left ejection is far in excess of any error which might arise from parallax, from differences in vibration frequency of the recording instruments, or from a delay in conduction of the pressure pulse (K41). The variability is due to the fact that the T wave and the end of ejection express the result of different types of stresses. The former is the resultant of the electrical stresses in the various regions of the heart. Its termination does not always mean that electrical stresses have entirely disappeared; but sometimes simply that the electrical stresses are balanced out in the line of the lead (K41). The end of ejection, as usually estimated, marks simply the time when the ventricle as a unit ceases to shorten and begins to lengthen, that is, when the algebraic sum of the contraction and relaxation processes in various regions is equal to zero (K41); certain fractions have probably begun to lengthen before this time, and certain others do not relax until some time afterwards. It would be surprising, therefore, to expect a constant relationship between the end of ejection in either ventricle and the period of unbalanced stresses marked by the T wave.

2. *The relation of the action current to the process of excitation and to the refractory phase.* Little attention has been paid to the relationship existing between the action current of the heart and the processes of excitation and refractoriness, and yet it seems that an evaluation of these relationships will go far in ultimately clarifying the interrelation of the various manifestations which in toto we call activity and sometimes loosely excitation. The early work of Burdon-Sanderson and Page (G13) and Tait (L56) indicated that the duration of the total refractory phase of the heart was about the same as that of its action current; and varied in the same direction when conditions were modified. Later

work by Trendelenburg (L58), de Meyer (L43) and Adrian (G1) showed, however, that the total refractory phase outlasted the action current. Trendelenburg (L58) observed a decrease in this difference as the temperature of the isolated heart preparation was elevated. Kroenecker and Stirling (cf. G1) noted that the total refractory phase persisted until mid-diastole when the temperature was low. Both Trendelenburg (L58) and de Boer (G6) found that veratrine and muscarine also caused the total refractory phase to outlast the action current for a considerable period of time.

It has been maintained by Adrian (G1) that the absolute refractory phase has practically the same duration as the action current. Holzlöhner (L27a) on the contrary has recently confirmed Trendelenburg's observations (L58) that the absolute refractory period is shorter than the action current. Mines (L45, I18) observed that the variations of the absolute refractory phase were in the same direction as those of the action current both when the rate of the heart was varied and when muscarine was injected.[6] (See addendum 3, p. 500.)

It is interesting to note in this connection that the decrease in electrical resistance which accompanies activity in the turtle's ventricle has been found to outlast the pressure changes (D61).

At present there is a tendency to consider the mechanical, electrical and excitatory phenomena discussed in this section as different aspects of the same underlying series of events (cf. p. 451). Their relation to

[6] After the review had gone to press, the reviewer obtained some facts which bear directly on this question. An inquiry was made among several colleagues to determine whether or not extrasystoles can start before the end of the T wave. Dr. R. W. Scott and Dr. E. C. Andrus informed me that they were unable to find such a case in a series of about 100 records and 300 records, respectively. Dr. R. Ashman, however, showed me such a record; Drs. H. Feil and M. Siegel found three such cases; and I have found three in my own series. In no instance did the initial deflection of the extrasystolic beat come before the peak of the T wave, although in one of my cases it arose very soon after. The only published record of this phenomenon seen by the reviewer is shown in figure 108 of Wenckebach and Winterberg's monograph (K7).

Dr. R. Ashman showed me several electrograms of the turtle heart, and an electrocardiogram of a dog, in which the T wave was interrupted by a premature initial deflection. A careful survey of my own collection of electrocardiograms of dogs showed a number of instances in which the T wave was interrupted by premature beats; and a survey of Dr. C. J. Wigger's collection revealed other similar records. In one instance in his collection the interruption actually occurred before the peak of the T wave. These facts indicate that the absolute refractory phase is over, at least occasionally, before the T wave is complete.

one another in the individual fractions of the ventricle is, however, still a matter of guesswork. It is impossible, furthermore, to differentiate any one phenomenon as the cause of any other. We can only say that they are all initiated by a process of excitation, and that the ultimate source of energy is chemical.

The evidence given in the preceding section has shown that the T wave was present in the electrocardiogram because the disappearance of the manifestations of the active state occurred asynchronously in the various parts of the heart (I 16, K4, K23, K30, K77, K82). Those authors who, like H. Straub (I 21, L54), W. Straub (L55), Zbzyzewski (L68) among others, have attributed the T wave to an anabolic chemical process, simply emphasize another aspect of the same theory, the effort being to correlate the facts with our chemical and physico-chemical knowledge (L54). It seems superfluous to assume at present, as Seemann (H6), A. Hoffmann (L26), Hoffmann and Selenin (L27), and Vaquez (L59) did, that a chemical mechanism producing contraction, distinct from the one accompanying excitation, is the cause of the T wave in the electrocardiogram.

CLINICAL ASPECTS OF THE SIGNIFICANCE OF THE T WAVE. 1. *Variations of the T wave with age.* Records so far obtained have failed to determine whether or not a T wave is present in the fetal electrocardiogram (M2, M4). According to Doxiades (M3), the electrocardiogram of the newborn infant has no T wave until breathing commences. Funaro and Nicolai (M5) found, on analyzing the electrocardiograms of forty-five children, that the T wave was small in the first month of life, but after that quickly reached adult proportions. Heubner (M6), Noeggerath (M8) and Hecht (M1) reached the same conclusion. Krumbhaar and Jenks (M7), on analyzing the electrocardiograms of forty-two infants and children, observed that a T wave was present at birth which decreased in size during the first week. The T wave was practically absent at the end of the first week, in all their cases; but after this period it increased rapidly. Inverted and diphasic T waves were noted by them in later childhood. Seham (M9) made an analysis of a still larger series of children, 103 cases in all. He found that the T wave was small or absent in the first ten days of life; and was inverted in 15 per cent of the cases in the early teens.

Linetzky (N53) made a statistical analysis of the electrocardiogram in adult life covering ages from twenty to seventy years. On arranging his 330 cases into ten-year groups he found that the T wave decreased in height with age. This is in accordance with the less extensive

analyses of Pribram and Kahn (N70), Strubell (N84), and Calandre (N17), who noted that the T wave was decreased in old age.

2. *The diagnostic significance of the T wave in the electrocardiogram.* The T wave of the normal human subject is usually upright in all three leads. It may, however, be diphasic or inverted in lead III (K8, N2). In man, the presence of an inverted T wave in either lead I or II, or in both, indicates some abnormality; in animals, such as the dog, an inverted T wave may be a normal occurrence in lead II (K41). At the outset of the clinical discussion two facts require emphasis: 1. An inverted T wave in leads I and II indicates some cardiac abnormality, though this abnormality is not necessarily organic or fatal. 2. Cardiac abnormalities similar to those having inverted T waves occur in which the T wave is upright. In other words, the absence of an inverted T wave is not as significant as its presence. The inverted T wave is to be considered as a sign, and should be correlated with the other evidence presented by the patient in making a diagnosis or prognosis (cf. K55, N33, N97). A small or inverted T wave has a different significance when it is associated with a normal QRS complex in all leads, than when it occurs with an abnormal initial deflection. It makes some difference also whether the abnormality of the initial deflection is one of size and direction only, or of duration as well.

Parallel changes in the QRS and T deflections, without a prolongation of the former may be caused by a shifting of the anatomical axis of the heart. The small upright or inverted T wave found in one or more leads during deep expiration (N30) and in the Valsalva experiment (N16, N30), and the large T wave occurring in pregnancy (N77), both of which are associated as a rule with similar changes in the QRS deflection, are caused by a shifting of the anatomical axis. This is also true in dextrocardia, in which the inverted T wave is associated, as a rule, with an inverted initial complex (K74, N3, N42, N60, N65). A shift in the anatomical axis may also be responsible, at least in part, for the changes of the T wave observed in preponderant hypertrophy (N9, N13, N53, N79, N83). (See addendum 4, p. 500.)

The changes in the QRS and T deflections, in thyroid disease, which are parallel to each other, have been ascribed to alterations outside the heart, namely, alterations in tissue resistance and skin capacity (J4, J7, N67) (cf. discussion p. 458). It is now recognized that large upright T waves, usually associated with large initial deflections, are generally present in Graves' disease (N17, N35, N36, N49, N51, N86, N99); after thyroidectomy and iodide medication the size of the deflections

decreases as the patient improves (N35, N36, N49). (Goodall and Rogers (N32) have reported a decrease in the height of the T wave in tachycardia accompanying Graves' disease.) On the other hand, small or absent T waves associated with small initial complexes are the rule in myxedema (J4, N67, N88, N100) and cretinism (N87); these waves increase in size as the patient improves under iodide or thyroid therapy (N87, N88, N100). The parallel changes in the QRS and T deflections found in edema, ascites, and obesity are also explained as a result of skin capacity changes (J7).

In a second group of inverted T waves are those in which the initial deflection is prolonged beyond the normal range, and oppositely directed to the T wave. This picture—and the reverse picture of an upright T wave and an inverted main deflection—, as is well known, occurs in ventricular extrasystoles (K4, K8, N2) and in blocks in one of the bundle branches (K4, K8, K82, N27, N58, N73, N74). Obviously, the opposite direction of the chief initial deflection and the T wave results from the delay in exciting certain large regions (K41, K80).

The presence of large positive and inverted T waves in extrasystoles, associated as they are with abnormally small contractions of the ventricles, would rule out the idea that the size of the T wave is related to the amplitude of contraction of the whole heart, or to the relative size of the contraction of the two ventricles. The proponents of this belief (K20, K39, K43, K73) have referred to the following evidence in support of their conception: 1, the change in the size of the T wave in thyroid disease, they claim, is parallel to the change in amplitude of contraction; 2, the variations in the amplitude of the T wave are almost proportionate to the changes in pulse pressure (N5, N91); 3, the T wave becomes smaller when failure of the heart occurs (N50, N70, N92); and 4, the T wave increases after exercise (N63, N84). The significance of this evidence diminishes when we realize that large T waves have been reported in failing hearts (N11), and that in experimental and clinical hypertension the T wave may be unchanged (K41, K45), decreased, or even inverted (N17, N53, N55, N64). (See addendum 5, p. 500.) Large (N78) as well as unchanged T waves (N46) have been described in moribund patients. After adrenalin, the T wave may become flattened or inverted (A5, K41, L4, N22); after hemorrhage, or after reduction in the filling of the ventricle, its change often is the reverse of the alteration in the amplitude of ventricular contraction (N10, N61). It would be contradictory to these last-mentioned facts to assert any quantitative relationship between amplitude of contraction and

height or direction of the T wave. It is more logical to assume that the parallelisms noted are accidental and depend upon the manner in which the three factors that we know modify the T wave—namely, the duration and order of excitation in the various fractions of the heart, the relation of the anatomical axis to the recording electrodes, and the magnitude of the tissue resistances and skin capacity—combine their effects (cf. also L10, N17).

The inversions of the T wave which have perhaps been stressed the most clinically are those which occur with no noticeable alterations in the amplitude and direction of the QRS complex. The appearance of this type of electrocardiogram in one or more leads has been reported in a number of conditions, such as gallop rhythm (N14), uremia and nephritis (L12, N2, N98), fevers (N2), hypertension (L12, K55), cardiac failure (N11, N50, N80), angina pectoris (N2, N29, N94),[7] the moribund state (N45, N46), thrombosis, sclerosis or experimental ligation of the coronary artery (N2, N12, N18, N20, N31, N37, N40, N41, N44, N53a, N57, N64, N69, N75, N81, N89, N95, K68),[8] infarction of the ventricle (N96), and myocardial involvement of various types (N2, N6, N7, N21, N23, N25, N26, N43, N47, N48, N54, N56, N62, N63a, N68, N72, N82, N86, N90, N97, N99). An inverted T wave has also been a constant finding in myocardial degeneration produced with the deep X-ray in dogs and sheep (N38a). It has been noted, furthermore, that the T wave in coronary involvement eventually may become upright again as the patient recovers (K68, N2, N69, N100). Similarly, in many cases of angina pectoris a return of the normal appearance of the T wave was noted with improvement of the case (N1); in some cases the inversion occurred and lasted only during the anginal attack (N29). The same return to normal has been reported after diphtheritic myocarditis (N82). Fahr (N28) has described a case of myxedema in which an inverted T wave became upright after treatment. Because of such observations, an inverted T wave has been regarded as a clew of organic involvement of the heart.

Some investigators, however, have observed cases with organic involvement of the heart in which the T wave was not inverted but flattened or entirely absent (K70, N2, N6, N11, N17, N21, N26, N37, N50, N52, N53, N64, N70, N86, N92). It is significant to note further that the T wave may be unchanged (N2, N11, N46, N52, N64, N68, N90, N94), or even larger than usual (N11, N64, N78, N79a) under these conditions.

[7] See also N45a.

[8] See also N11a, N95a.

Inversions of the T wave have, furthermore, been reported after the administration of drugs such as quinidine (K42a, N2), various members of the digitalis group (K12, K13, K18, K50, K54, K56, K59, K60, K65, K69, N2, N66), epinephrin (A5, K41, K69, K78, L4) and insulin (K36, K44, K51, K61, K83). Similarly, inversions, or flattening of the T wave have been noted after decreasing the O_2 tension of the alveoli (N34) or changing its CO_2 tension (N76). In the case of drug therapy, as in organic conditions, the effect is not constant; in some instances in fact the T wave even increased in amplitude (K12, K13, K18, K41, K42a, K60, K69, K84, N66).

The observations summarized above indicate that an inverted T wave is not pathognomonic of any clinical entity, but is rather to be regarded as the result of a disturbance in the pathway of retreat, which simulates persistence of activity in the apical region of the left ventricle,—unless its cause can be shown to be a shifted anatomical axis or a changed capacity of the skin. When unassociated with any change in amplitude or direction of the initial deflection it is probably caused by a delayed offset of activity somewhere in this general region which results from a delayed onset, from a relative prolongation of activity, or from both. It tells nothing about the cause. Similarly, an upright T wave simply shows that this apparent simulation of persistence of activity in the apical region of the left ventricle does not occur. It does not tell whether the heart is normal or the seat of organic involvement. The inversion of the T wave is, therefore, a localizing sign, in the same way that a prolongation of the QRS complex is considered evidence of block somewhere in the course of the bundle or its branches. This conclusion is the only one which correlates the clinical observations with the current opinion of the cause of the T wave. It is directly supported by the following facts: 1. The direction of the T wave in ventricular extrasystoles is determined by the location of the ectopic focus (K4, K41). 2. In bundle branch block, the direction of the T wave is determined by the bundle branch involved (K4, K8, K82, N27, N58, N73). 3. The modifications in the T wave which occur with variations of temperature are dependent on the location of the temperature change (I8, K10, K34, K67, K80, K82). 4. Inverted T waves are found when the left apical regions of the heart are the seat of infarction (N96), and occur in coronary involvement apparently only when the left coronary artery is affected either clinically or experimentally (K40, K68, N31, N81). The reviewer is not aware of any uncomplicated clinical case of thrombosis, sclerosis, or embolism involving the right coronary artery which

shows an inverted T wave in leads I and II. Until evidence is produced to the contrary, the theory of localized involvement of the heart—whether this process be organic or functional does not matter—can be tentatively accepted as the one best able to explain the clinical appearance of an inverted T wave without apparent change in the QRS complex. (See addendum 6, p. 500.)

Another interesting deviation of the T wave may be mentioned, namely, a fusion of this wave with the R wave, which resembles the double summit type of record sometimes obtained on leading off directly from injured-uninjured areas. Such electrocardiograms have been described by Smith (K68) in experimental ligation of the left coronary artery, directly following the application of the ligature. It has also been noted clinically, soon after the appearance of coronary thrombosis and occlusion (N18, N44, N69, N95).[9] It is not pathognomonic of coronary involvement, for it has also been observed in rheumatic myocarditis (N23), in the moribund state (N71)[10] after the administration of digitalis (N24), in experimental infarction of the heart (N18a), in massive injury of the heart (K27), and in anaphylactic reactions (K42). It would seem that this type of electrocardiogram indicates the throwing out of action, either completely or incompletely, of a large area of the heart. In most cases of coronary involvement that recover, the T wave becomes inverted for a time before finally becoming upright in the normal manner (N44, N45a, N69, N79a).

3. *The prognostic significance of the T wave in the electrocardiogram.* An inverted T wave is so often associated with organic heart disease that it has been regarded as a basis of grave prognosis (N2, N4, N11, N15, N17, N19, N38, N39, N40, N46, N47, N55, N59, N68, N80, N84, N85, N93). Willius found (N93), on making a statistical study of the life expectancy of patients with inverted T waves, that the average duration of life was shorter in these cases than in the control series where the T wave was upright. A fatal prognosis in a given case cannot be made, however, from an inverted T wave alone, because, as we have shown above (p. 473), the inversion may be present as a result of drug medication, or as a manifestation of a temporary non-fatal cardiac abnormality. Furthermore, it does not follow that an upright T wave precludes a fatal prognosis because an upright T wave has been found even in moribund patients (N46, N78). In other words, the direction and size of the T wave in itself is of little prognostic value in individual cases, and may in

[9] See also N79a.

[10] See also N24a, N55a.

fact be misleading. The value of an inverted T wave in working out actuary figures of life expectancy is not to be denied, but the value of applying the results to a given case is questionable.

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D 70. Tschachotin, S. Pflüger's Arch., 1907, cxx, 565 (oriented molecules as a cause of tissue electricity).
D 71. Tschagowetz, W. J. Zeitschr. f. Biol., 1908, l, 247 (semipermeable membrane and tissue currents).
D 72. Verworn, M. Pflüger's Arch., 1917, clxvii, 289 (artificial model for production of potential differences).
D 73. Verzár, F. Pflüger's Arch., 1912, cxliii, 252 (effect of temperature on tissue potential).
D 74. Vorschütz, J. Pflüger's Arch., 1921, clxxxix, 181 (nature of membrane).
D 75. Waller, A. D. Journ. Physiol., 1909, xxxviii, xliii (effect of temperature on tissue potential).
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D 77. Weiss, H. Pflüger's Arch., 1922, cxciv, 152 (effect of electric current on cell).

See also: Lewis (K4), 58–61 (membrane theory); Adrian (G1, G2) (membrane theory).

E. Positive wave in auricle

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E 2. Garrey, W. E. Amer. Journ. Physiol., 1924, lxviii, 128.
E 3. Gaskell, W. H. Journ. Physiol., 1886, vii, 451.
E 4. Meek, W. J. and J. A. E. Eyster. Amer. Journ. Physiol., 1913, xxx, 271.
E 5. Samojloff, A. Pflüger's Arch., 1923, cxcix., 579.

F. Positive wave in nerve

F 1. Garten, S. Pflüger's Arch., 1910, cxxxvi, 545.
F 2. Sochor, N. Zeitschr. f. Biol., 1912, lviii, 1.
F 3. Tigerstedt, C. and S. Donner. Skand. Arch. f. Physiol., 1913, xxx, 309.
F 4. Verzár, F. Pflüger's Arch., 1926, ccxi, 244.
F 5. Woronzow, D. S. Pflüger's Arch., 1924, ccvi, 1.

G. Action current recorded from injured-uninjured tissue lead offs (the monophasic action current of heart)

G 1. Adrian, E. D. Journ. Physiol., 1921, lv, 193 (source of distortion of monophasic action current).
G 2. Adrian, E. D. Journ. Physiol., 1925, lx, 301 (contour of monophasic action current and cause of its distortion).
G 3. Adrian, E. D. and D. R. Owen. Journ. Physiol., 1921, lv, 326 (tendon lead off not equivalent to injured region lead off).
G 4. Bishop, G. H., J. Erlanger and H. S. Gasser. Amer. Journ. Physiol., 1926, lxxviii, 592 (source of distortion of action current recorded with direct leads).
G 5. Bishop, G. H. and A. S. Gilson, Jr. Amer. Journ. Physiol., 1927, lxxxii, 478 (source of distortion in action current recorded with direct leads).
G 6. de Boer, S. Journ. Physiol., 1915, xlix, 324 (contour of monophasic action current).
G 7. Bohnenkamp, H. Pflüger's Arch., 1923, cci, 131 (contour of monophasic action current; effect of nerves on its duration; variation of its duration in different regions).
G 8. Boruttau, H. Arch. f. Physiol., 1913, 519 (source of distortion of monophasic action current; effect of vagus nerve and of muscarine on monophasic action current recorded from different regions; variation in duration and contour of monophasic action current in different regions).
G 9. Broemser, P. Zeitschr. f. Biol., 1923, lxxviii, 139 (contour of monophasic action current at various points along nerve; method of correcting string galvanometer records).
G 10. Buchanan, F. Journ. Physiol., 1927, xliv, ii (use of KCl to obtain monophasic action currents).
G 11. Burdon-Sanderson, J. Journ. Physiol., 1895, xviii, 117 (contour of monophasic action current and source of its distortion).
G 12. Burdon-Sanderson, J. Journ. Physiol., 1898, xxiii, 325 (effect of temperature on duration of monophasic action current).

G 13. Burdon-Sanderson, J. and P. J. M. Page. Journ. Physiol., 1880, ii, 384; 1883, iv, 327 (effect of temperature changes and of injury on duration of monophasic action current).

G 14. Davis, H. and D. Brunswick. Amer. Journ. Physiol., 1926, lxxv, 497 (accuracy of string galvanometer records; distortion of action currents recorded with direct leads).

G 15. Drury, A. N. and G. R. Brow. Heart, 1926, xii, 321 (method of calculating monophasic action current; extrinsic effects; sources of distortion in calculated monophasic action current).

G 16. Erlanger, J. and H. S. Gasser. Amer. Journ. Physiol., 1924, lxx, 624 (a source of distortion of monophasic action current of nerve recorded away from point of stimulation).

G 17. Gasser, H. S. and J. Erlanger. Amer. Journ. Physiol., 1922, lxii, 496 (accuracy of string galvanometer records).

G 18. Gilson, A. S., Jr. Amer. Journ. Physiol., 1927, lxxxi, 479 (contour of monophasic action current at site of stimulation).

G 18a. Gilson, A. S., Jr. Amer. Journ. Physiol., 1927, lxxxii, 533 (effect of injury and fatigue on monophasic action current recorded at site of stimulation).

G 19. Hermanns, L. Zeitschr. f. Biol., 1912, lviii, 261 (contour of monophasic action current).

G 20. Hofmann, F. B. Zeitschr. f. d. gesammt. exper. Med., 1920, xi, 156; 1926, l, 130; Deutsch. med. Wochenschr., 1926, lii, 15 (duration of monophasic action current during vagus stimulation and during cardiac acceleration; duration of monophasic action current in different localities).

G 21. Holzlöhner, E. Zeitschr. f. Biol., 1927, lxxxv, 585 (contour of monophasic action current; effect on it of KCl and $CaCl_2$ when applied in different regions).

G 22. Jolly, W. A. Quart. Journ. Exper. Physiol., 1916, ix, 9 (contour of monophasic action current; significance of preliminary dip in it).

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G 26. Rosenberg, H. Pflüger's Arch., 1927, ccxvi, 300 (contour of monophasic action current of nerve at different distances from point of excitation).

G 27. Rümke, H. C. Arch. néerl. de physiol., 1918, i, 161, 682 (contour of monophasic action current; its duration in different regions).

G 28. Samojloff, A. Arch. f. Physiol., 1906, Supp. 217 (effect of temperature on duration of monophasic action current).

G 29. Samojloff, A. Pflüger's Arch., 1914, clv, 471 (cause of distortions of monophasic action current; variations in its duration and gradients).

G 30. Veen, H. Arch. néerl. de sc. naturw., Ser. iiiB, 1915, ii, 284 (quoted by Yoshida, G33) (contour of monophasic action current).

G 31. Wiechmann, E. Zeitschr. f. Biol., 1922, lxxiv, 81 (contour of monophasic action current).

G 32. Woronzow, D. S. Zeitschr. f. Biol., 1927, lxxxvi, 231 (extrinsic effects in monophasic action current).

G 33. Yoshida, H. Zeitschr. f. Biol., 1926, lxxxiv, 51 (distortions of monophasic action current; effect of temperature, acidity, injury and constant current on its contour and duration).

G 34. Yoshida, H. Zeitschr. f. Biol., 1926, lxxxiv, 453 (duration of monophasic action current at apex and base).

See also: Arbeiter (L2) (distortions of monophasic action current); Bayliss and Starling (K10) (duration of monophasic action current in different regions); de Boer (I2) (contour of monophasic action current); Clement (I4) (effect of tissue shunting on recorded potential), Lucas (D48) (effect of tissue shunting on recorded potential); Mines (I16); Samojloff (I19) (direct and indirect leads); Schellong (J6) (effect of size of electrodes on contour of record, direct and indirect leads); Seemann (H6) (effect of tissue shunting on recorded potential). See addendum 1.

H. Theories of dualistic origin of action current of heart

H 1. Athanasiu, J. Journ. de physiol. et path. gén., 1924, xxii, 1; Compt. rend. Acad. d. sci., 1923, clxxvii, 783, 1070 (neurogenic-myogenic theory).

H 2. de Boer, S. Zeitschr. f. Biol., 1915, lxv, 428 (fibrillary-sarcoplasmic theory).

H 3. Fredericq, H. Arch. intern. de physiol., 1912, xi, 253; 1921, xviii, 67; Compt. rend. Soc. de biol., 1921, lxxxv, 242 (fibrillary-sarcoplasmic theory).

H 3a. Herzog, F. Deutsch. Arch. f. klin. Med., 1927, clv, 294 (initial deflection caused by excitation, final by contraction).

H 4. de Meyer, J. Arch. intern. de physiol., 1919, xv, 151; 1921, xvi, 44, 65, 172, 193; 1921–1922, xvii, 1; Arch. néerl. de physiol., 1922, vii, 292; Compt. rend. Soc. de biol., 1920, lxxxiii, 308, 1217 (initial phase due to excitation, final to a deformation caused by sarcoplasmic activity).

H 5. Rossi, A. La malattie del cuore, 1922, 281 (abstracted in Arch. d. mal. du coeur, 1923, xvi, 301) (inadequacy of evidence in embryonic heart tissue for fibrillary-sarcoplasmic theory).

H 6. Seemann, J. Zeitschr. f. Biol., 1913, lix, 53 (initial phase due to excitation, final accompanies contraction).

See also: de Boer (G6) (I 2) (fibrillary-sarcoplasmic theory); Eppinger and Rothberger (K27) (circular-longitudinal fibre theory); Fredericq (K1) (fibrillary-sarcoplasmic theory); Henrijean (K37) (modified excitation-contraction theory); Hoffmann (K39); Hoffmann (L26), Hoffmann and Selenin (L27) (excitation-contraction theory); Kraus (K43) (two types of muscle combine to cause form of electrocardiogram); Samojloff (G29) (criticism of theories of dual origin); Rümke (G27) (fibrillary-sarcoplasmic theory); Veen (G30) (fibrillary-sarcoplasmic theory); Wiechmann (G31) (criticism of theories of dual origin); Wertheim-Salomonson (B25) (evidence in chick embryo for fibrillary-sarcoplasmic theory); Woronzow (G32) (I23) (I24) (criticism of theories of dual origin.

I. The T wave in the electrogram

I 1. Bayliss, W. M. and E. H. Starling. Journ. Physiol., 1891, xii, xx (effect of temperature on electrogram of mammal).

I 2. de Boer, S. Pflüger's Arch., 1919, clxxiii, 78; Arch. néerl. de physiol., 1918, iii, 7; Amer. Journ. Physiol., 1925, lxxiv, 158 (interference theory; effect of digitalis on T wave).

I 3. Brandenburg, K. and P. Hoffmann. Med. Klin., 1911, 39 (effect of digitalis on T wave).

I 4. Clement, E. Zeitschr. f. Biol., 1912, lviii, 110 (T wave in differential electrogram).

I 5. Dale, D. and G. R. Mines. Journ. Physiol., 1913, xlvi, 319 (effect of cardiac nerves on T wave; interference theory).

I 6. Erfmann, W. Zeitschr. f. Biol., 1913, lxi, 155 (T wave in differential electrogram).

I 7. Freud, J. Journ. de physiol. et path. gén., 1926, xxiv, 725 (T wave due to excitation of aortic bulb).

I 8. Garten, S. and W. Sulze. Zeitschr. f. Biol., 1916, lxvi, 433 (T wave in differential electrogram; effect of temperature on it; interference theory).

I 9. Gotch, F. Proc. Roy. Soc., 1907, B lxxix, 323; Heart, 1909–1910, i, 235 (T wave due to return of excitation to base).

I 10. Henle, K. Zeitschr. f. Biol., 1911, lv, 295 (effect of applying a constant current at base or apex on T wave).

I 11. Kraus, F. and G. F. Nicolai. Zentralbl. f. Physiol., 1907, xxi, 678 (T wave due to return of excitation to base).

I 12. Leontowitsch, A. Pflüger's Arch., 1912, cxlvii, 473 (effect of Ca on T wave).

I 13. Lewis, T. Phil. Trans. Roy. Soc., 1916, B ccvii, 221 (spread of excitation in ventricle of tortoise).

I 14. Meek, W. J. and J. A. E. Eyster. Amer. Journ. Physiol., 1912, xxxi, 31 (inadequacy of Gotch's conduction theory).

I 15. Meyer, F. Pflüger's Arch., 1927, ccxvi, 765 (mechanical model for working out interference theory).

I 16. Mines, G. R. Journ. Physiol., 1913, xlvi, 188, 349 (interference theory; effect of temperature and pH changes on T wave).

I 17. Mines, G. R. Journ. Physiol., 1913–1914, xlvii, 419 (effect of vagus on T wave).

I 18. Mines, G. R. Journ. Pharm. Exper. Therap., 1913–1914, v, 425 (effect of muscarine on T wave).

I 19. Samojloff, A. Pflüger's Arch., 1910, cxxxv, 417 (effect of vagus on T wave).

I 19a. Samojloff, A. Pflüger's Arch., 1927, ccxvii, 583 (effect of vagus and "vagus hormone" on T wave).

I 20. Spurk, P. Zeitschr. f. d. gesammt. exper. Med., 1926, l, 125 (interference theory worked out with mechanograms of apex and base).

I 21. Straub, H. Zeitschr. f. Biol., 1910, liii, 499 (effect of temperature changes and of fatigue on T wave).

I 22. Straub, W. Arch. f. Exper. Path. u. Pharm., 1915, lxxix, 19 (effect of digitalis on T wave of auricle).

I 23. Woronzow, D. S. Zentralbl. f. Physiol., 1914, xxviii, 305 (effect of location of lead offs on T wave).

I 24. Woronzow, D. S. Pflüger's Arch., 1915, clx, 581 (effect of location of lead offs on T wave; interference theory; inadequacy of theories of dual origin).

See also: Bayliss and Starling (K10) (interference theory); Bohnenkamp (G7) (interference theory); Boruttau (G8) (interference theory); Burdon-Sanderson (G12) (interference theory); Burdon-Sanderson and Page (G13) (interference theory); Gilson (G18a) (interference theory); Hofmann (G20) (interference theory); Holzlöhner (G21) (interference theory); Jolly (G22) (interference theory); Katz and Weinman (K41) (duration of action current in adjacent regions); Klewitz (L37) (effect of temperature on T wave); von Kries (G24) (interference theory); Lewis and Rothschild (K48); Rümke (G27) (interference theory); Samojloff (G28) (G29) (interference theory); Tschernorutzky (K72); Waller (K75); Wiggers (K79); Woronzow (G32) (interference theory); Yoshida (G33) (G34) (interference theory).

J. The difference between direct and indirect lead offs

J 1. Boden, E. and P. Neukirch. Pflüger's Arch., 1918, clxxi, 146 (relation of direct to indirect lead offs in isolated mammalian hearts).

J 2. Einthoven, W. Arch. intern. de physiol., 1906–1907, iv, 132 (the three standard leads).

J 3. Einthoven, W. and J. Bijtel. Pflüger's Arch., 1923, cxcviii, 439; Arch. néerl. de physiol., 1922, vii, 407 (effect of conditions external to heart on the contour of the electrocardiogram).

J 4. Lueg, W. Pflüger's Arch., 1926, ccxii, 649; Zeitschr. f. klin. Med., 1926, civ, 337 (effect of myxedema before and after thyroidectomy on skin capacity).

J 5. Mayer, K. Wien. klin. Wochenschr., 1914, 1139 (lead offs from bath in which person is immersed).

J 6. Schellong, F. Zeitschr. f. d. gesammt. exper. Med., 1926, 1, 488 (relation of direct to indirect lead offs; effect of location of lead offs on contour of electrocardiogram; artificial model for testing this effect).

J 7. Wilson, F. N., S. W. Wishart and G. R. Herrmann. Proc. Soc. Exper. Biol. and Med., 1926, xxiii, 276 (effect of changes in the resistance of tissues external to heart on electrocardiogram; effect of ascites and edema on electrocardiogram).

J 8. Yoshida, H. Zeitschr. f. Biol., 1926, lxxxiv, 478 (relation of direct to indirect lead offs).

See also: Clement (I 4) (effect of location of lead offs on the contour of the electrocardiogram; artificial model for testing this effect); Lewis (I 13) (relation of direct to indirect lead offs); Nobel, Rosenblüth and Samet (N67) (effect of skin resistance changes on electrocardiogram); Samojloff (I 19) (direct and indirect leads).

K. The T wave on the electrocardiogram

K 1. Fredericq, H. Aspects actuels de la physiologie du myocarde, Le Presses Univ. de France, Paris, 1927, 206–243 (review of literature on T wave).

K 2. Kahn, R. H. Ergebn. d. Physiol., 1914, xiv, 216–239 (review of literature on T wave).

K 3. Kraus, F. and G. F. Nicolai. Das Elektrokardiogram des Gesunden und Kranken Menschen, Veit. u. Co., Leipsig, 1910 (conduction theory).

K 4. Lewis, T. The mechanism and graphic registration of the heart beat, 3rd ed., Shaw & Sons, London, 1925, 98, 102–104 (theory of distributed vs. theory of limited potential differences); 116–122 (meaning of T wave).

K 5. Tigerstedt, R. Die Physiologie des Krieslaufes, 2. auflage, Verein Wissensch. Verl., Berlin and Leipsig, 1921, ii, 206–269 (review of literature on T wave).

K 6. Weber, A. Die Elektrokardiographie, J. Springer, Berlin, 1926, 85–110 (theory of distributed potential; T wave an expression of unequal cessation of activity).

K 7. Wenckebach, K. F. and H. Winterberg. Die Unregelmässige Herztatikeit, W. Engelmann, Leipzig, 1927, 69–73 (development of potential in muscle strips, indirect leads); 98–102 (T wave in electrocardiogram).

K 8. Wiggers, C. J. Circulation in health and disease, 2nd ed., Lea & Febiger, Philadelphia, 1923, 269–279 (T wave in electrocardiogram).

K 9. Bayliss, W. M. and E. H. Starling. Proc. Roy. Soc., 1892, l, 211 (longer duration of activity at base as cause of T wave).

K 10. Bayliss, W. M. and E. H. Starling. Internat. Monatschr. f. Anat. u. Physiol., 1892, ix, 256 (longer duration of activity at base as cause of T wave; effect of temperature changes and of injury on T wave).

K 11. Beeke, W. G., S. Johnson and H. S. Harris. Amer. Heart Journ., 1927, ii, 302 (effect of experimental lesions of left ventricular base on T wave).

K 12. Bickel, A. and A. Tsividis. Biochem. Zeitschr., 1912, xlv, 462 (effect of digitalis on T wave).

K 13. Bickel, A. and M. Pawlow. Biochem. Zeitschr., 1913, xlviii, 459 (effect of strophanthus on T wave).

K 14. de Boer, S. Amer. Journ. Physiol., 1925, lxxiv, 158 (criticism of theory of limited potential differences).

K 15. de Boer, S. Amer. Journ. Physiol., 1926, lxxvi, 1 (ditto as above, indirect leads).

K 16. Bürger, M. and M. Baur. Zeitschr. f. d. gesammt. exper. Med., 1925, xliv, 568 (effect of Ringer solution, poor in sugar content, on T wave of isolated heart, indirect leads).

K 17. Cohn, A. E. and F. R. Fraser. Journ. Pharm. Exper. Path., 1913–1914, v, 512 (effect of digitalis on T wave).

K 18. Cohn, A. E., F. R. Fraser and R. A. Jamieson. Journ. Exper. Med., 1915, xxi, 593 (effect of digitalis on T wave).

K 19. Craib, W. H. Skand. Arch. f. Physiol., 1926, xlix, 110; Heart, 1927, xiv, 71 (doublet theory).

K 20. Daly, I. de B. Proc. Roy. Soc., 1924, B xcv, 279 (T wave considered a measure of the relative work of the two ventricles).

K 21. Eiger, M. Pflüger's Arch., 1913, cli, 1 (orientation of ions during recovery as cause of T wave).

K 22. Eiger, M. Pflüger's Arch., 1915, clxii, 433 (as above; electrocardiogram as a biogram).

K 23. Einthoven, W. Pflüger's Arch., 1908, cxxii, 517 (persistence of activity at base as cause of T wave in lead III, persistence in right heart as cause of T wave in leads I and II).

K 24. Einthoven, W. Pflüger's Arch., 1913, cxlix, 65 (as above).

K 25. Einthoven, W. Arch. néerl. de physiol., 1924, ix, 406 (heart cells as electrical units).

K 26. Einthoven, W., G. Fahr and A. de Waart. Pflüger's Arch., 1913, cl, 275 (electrical axis).

K 27. Eppinger, H. and C. J. Rothberger. Wien. klin. Wochenschr., 1909, xxii, 1091; Zentralbl. f. Physiol., 1910, xxiv, 1053 (effect of lesions of ventricle on T wave; theory of dual origin).

K 28. Eyster, J. A. E. and W. J. Meek. Heart, 1912–1913, iv, 59 (effect of atropine and morphine on T wave).

K 29. Eyster, J. A. E. and W. J. Meek. Arch. Int. Med., 1913, xi, 204 (literature on T wave; theory of distributed potential differences).

K 30. Fahr, G. Arch. Int. Med., 1920, xxv, 146 (theory of distributed potential differences).

K 31. Fahr, G. and A. Weber. Deutsch. Arch. f. klin. Med., 1914, cxvii, 361 (theory of distributed potential differences).

K 32. Freud, J. and Saddi-Nazim. Jour. de physiol. et path. gén., 1927, xxv, 500 (effect of histamine and arsenates on T wave).

K 33. Frey, W. and H. Quincke. Zeitschr. f. d. gesammt. exper. Med., 1926, xlix, 447 (effect of K and Ca on T wave).

K 34. Ganter, G. Deutsch. Arch. f. klin. Med., 1913, cxii, 559 (effect of local temperature changes on T wave).

K 35. Gonzalo, G. R. Siglo Med., 1925, lxxvi, 528 (T wave as evidence of activity of conus).

K 36. von Haynal, E. Berl. klin. Wochenschr., 1925, i, 403; ii, 1729 (effect of insulin on T wave).

K 37. Henrijean, F. Compt. rend. Acad. d. sci., 1925, clxxxi, 619; 1925, clxxx, 1703; 1926, clxxxiii, 671 (effect of caffein and adrenalin on T wave; theory of dual origin).

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K 39. Hoffmann, A. Deutsch. med. Wochenschr., 1912, xxxviii, 1531 (T wave as measure of relative strength of two ventricles).

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See also: BAYLISS (D1); BARTOS AND BURSTEIN (L4) (effect of epinephrin and of aortic compression on T wave); CLOUGH (N22); GARTEN AND SULZE (I8)

(theory of distributed potential differences; effect of temperature on T wave); GILSON (G18a) (criticism of Craib's results and of de Meyer's theory); GOLD, DE GRAFF AND EDWARDS (N31); GOTCH (I9) (conduction theory); JOLLY (G22); KRAUS AND NICOLAI (I11); KRAUS, NICOLAI AND MEYER (A5) (effect of adrenalin on T wave); LEWIS (I13); LILLIE (D8, D9); DE MEYER (H4); NICOLAI AND SIMONS (N66); PIPER (L48) (asynchronism of two ventricles as cause of T wave); RYSER (N76); STRAUB (I 21) (effect of temperature on T wave); YOSHIDA (J8) (distributed potential theory). See section H for references to theories of dual origin. See addendum 2.

L. RELATION OF THE T WAVE TO THE REFRACTORY PERIOD AND TO THE MECHANICAL AND CHEMICAL EVENTS

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L 3. BABKIN, B. P. Pflüger's Arch., 1908, cxxv, 595 (duration of monophasic action current and of contraction in red and white muscle).

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See also: Burdon-Sanderson (A1), 443–448 (time relation between electrical and mechanical events); Fredericq (K1), 179–186 (relation between electrical and mechanical events); Fulton (D6), 224–233 (relation between electrical and mechanical events); Kahn (K2), 68–70, 79–90 (time relations of T wave); Lewis (K4), 47–53 (relation between electrical and mechanical events); Tigerstedt (K5), 238–240 (time relation between mechanical and electrical events); Wiggers (K8), 267–268 (time relation between electrical and mechanical events).

Adrian (G1) (relation of action current to refractory phase); Adrian (G2) (comparison of electrical and mechanical events in heart and skeletal muscle); Athanasiu (H1) (time relation between electrical and mechanical events); Bishop and Gilson (G5) (time relation between electrical and mechanical events in skeletal muscle; occurrence of "T wave" in electromyogram); de Boer (G6) (effect of veratrine and muscarine on time relation of action current to refractory phase); Bohnenkamp (G7) (time relation between electrical and mechanical events); Boruttau (G8) (as above); Burdon-Sanderson (G12) (duration of monophasic action current in heart and skeletal muscle); Burdon-Sanderson and Page (G13) (time relation of action current to refractory phase); Einthoven (K23); Einthoven (K24) (time relation of T wave to carotid pulse); Fahr (K30); Henle (I 10); Hering (K38) (persistence of action current after contraction has disappeared); Hofmann (G20) (effect of different conditions on time relation between electrical and mechanical events); Holzlöhner (G21) (time relation between electrical and mechanical events); Katz and Weinman (K41) (asynchronism of ejection of two ventricles; relation of T wave to right and left ventricular pressure pulses, and to aortic and pulmonary arterial pressure pulses, under different conditions; meaning of end of ejection); de Meyer (H4) (time

relation of T wave to contraction and relaxation); MINES (I16) (presence of action current after contraction has disappeared); MINES (I18) (relation of action current to refractory period); RAPPORT AND RAY (D61); SAMOJLOFF (G29) (time relation between electrical and mechanical events); SEEMANN (H6) (relation of action current to chemical changes); STRAUB (I21) (relation of action current to chemical changes; presence of action current when contraction has disappeared); USOFF (K73) (time relation of T wave to venous pulse); WEBER (K77); WILSON AND HERRMANN (K82) (time relation of action current to refractory phase of two ventricles); WORONZOW (I24) (time relation between electrical and mechanical events). See addendum 3.

M. EFFECT OF AGE ON T WAVE IN ELECTROCARDIOGRAM OF MAN

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M 2. CREMER, M. Münch. med. Wochenschr., 1906, 811 (fetal electrocardiogram).

M 3. DOXIADES, L. Berl. klin. Wochenschr., 1926, i, 303 (electrocardiogram of newborn infants).

M 4. FÒA, C. Arch. ital. de biol., 1911, lvi, 145 (fetal electrocardiogram).

M 5. FUNARO AND NICOLAI. Zentralbl. f. Physiol., 1908, xxii, 58 (electrocardiogram of early childhood).

M 6. HEUBNER, O. Monatschr. f. Kinderh., 1908, vii, 6 (electrocardiogram of young children).

M 7. KRUMBHAAR, E. B. AND H. H. JENKS. Heart, 1915–1917, vi, 189 (electrocardiogram in early childhood).

M 8. NOEGGERATH, C. T. Zeitschr. f. Kinderh., 1913, vi, 396 (electrocardiogram of children).

M 9. SEHAM, M. Amer. Journ. Dis. Child., 1921, xxi, 247; Abt's Pediatrics, Saunders Co., Philadelphia, 1924, iv, 282 (electrocardiogram in children).

See also: WIGGERS (K8), 287–288 (T wave in childhood); CALANDRE (N17) (T wave in old age); PRIBRAM AND KAHN (N70) (T wave in old age); LINETZKY (N53) (T wave at different adult ages); STRUBELL (N84) (T wave in old age).

N. CLINICAL ASPECTS OF THE SIGNIFICANCE OF THE T WAVE

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N 3. ABRAHAMSON, L. Quart. Journ. Med., 1924–25, xviii, 335 (T wave in dextrocardia).

N 4. ARRILLAGA, M. F. C. Bull. et mém. Soc. méd. h. hôp. de Paris, 1924, xlviii, 1493 (prognostic significance of an inverted T wave).

N 5. AVARITSIOTIS, E. Arch. d. mal. du coeur, 1926, xix, 705 (amplitude of T wave parallels the variations in height of oscillometric curve).

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N 7. Barker, L. F. Bull. Johns Hopkins Hosp., 1910, xxi, 358 (significance of an inverted T wave).

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N 10. Benjamin, K. Zentralbl. f. Herz. u. Gefässkr., 1924, xvi, 51 (effect of filling and of volume stroke of ventricle on T wave).

N 11. Beuttenmüller, H. Zentralbl. f. Herz. u. Gefässkr., 1919, xi, 145, 165 (effect of fever and of nephritis on T wave; effect of heart failure on T wave).

N 11a. Birchard, C. C. Can. Med. Assoc. Journ., 1928, xviii, 421 (inverted T wave in coronary thrombosis).

N 12. Bloedorn, W. A. and L. J. Roberts. Arch. Int. Med., 1923, xxxii, 718 (case report of left coronary occlusion with inversion of T wave).

N 13. Bock. Deutsch. med. Wochenschr., 1910, 633 (T wave in ventricular hypertrophy).

N 14. Bordet, E., J. Yacoël and R. Giroux. Arch. d. mal. du coeur, 1923, xvi, 205 (inversion of T wave in gallop rhythm).

N 15. Bosco, G. Revista Med. Latino-Amer., 1925, x, 1107 (prognostic significance of an inverted T wave).

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N 27. Eppinger, H. and O. Stoerk. Zeitschr. f. klin. Med., 1910, lxxi, 157 (T wave in bundle branch lesions).

N 28. Fahr, G. Amer. Heart. Journ., 1927, iii, 14 (inverted T wave in myxedema; effect of therapy on T wave in this condition).

N 29. Feil, H. S. and M. L. Siegel. Amer. Journ. Med. Sci., 1928, clxxv, 252 (T wave in an attack of angina pectoris).

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See also: Fredericq (K1), 196–201; Lewis, (K4), 161–165, 211 (T wave in extrasystoles of ventricular origin and in bundle branch blocks); Wenckebach and Winterberg (K7), 361–363; Wiggers (K8), 290–291, 294; Bayliss and Starling (K10); Bartos and Burstein (L4) (effect of adrenalin on T wave); Bickel and Tsividis (K12); Bickel and Pawlow (K13); Boruttau (L10) (relation of T wave to cardiac strength); Burgsch and Blumenfeldt (L12) (T wave in nephritis and in hypertension); Cohn, Fraser and Jamieson (K18); Daly (K20); Eppinger and Rothberger (K27); Ganter (K34); Garten and Sulze (I 8); v. Haynal (K36); Hoffmann (K39); Kahn (K40); Katz and Weinman (K41) (inverted T wave in normal anesthetized dogs); Koenigsfeld and Oppenheimer (K42) (fusion of R and T waves in anaphylaxis); Korns (K 42a); Kraus (K43) (T wave measures the strength of ventricular contraction); Kraus, Nicolai and Meyer (A5) (effect of epinephrin on T wave); La Barre (K44); Langley et al. (K45); Lueg (J4); McCullouch and Rupe (K50); Okashima and Okhuni (K51); Planelles and Werner (K54); Rehfisch (K55) (diagnostic value of an inverted T wave); Robinson and Wilson (K56); Rothberger and Winterberg (K59); Samet and Tezner (K60); Schäffer et al.

(K61); SELENIN (K65); SMITH (K67); SMITH (K68); STRAUB (K69); STRUBELL (K70) (T wave changes in moribund patients); USOFF (K73) (inverted T wave in patent ductus arteriosus); WALLER (K74) (T wave in dextrocardia); WHITEHEAD AND ELLIOTT (K78); WILSON AND FINCH (K80); WILSON AND HERRMANN (K82) (T wave in bundle branch block); WILSON, WISHART AND HERRMANN (J7); WITTGENSTEIN AND MENDEL (K83); YACOËL AND PAPAPANAYOTOU (K84). See addenda 4, 5 and 6.

ADDENDA

1. Woronzow (G32) observed a similar phenomenon in the frog's ventricle. F. Schellong (Zeitschr. f. d. gesammt. exper. Med., 1928, lxi, 278) demonstrated the presence of extrinsic effects in the monophasic action current of frog's ventricle strips by varying the position of block employed to render the tissue under one electrode inactive.

2. W. C. Buchbinder (Amer. Journ. Physiol., 1928, lxxxv, 356) noted an inversion of the T wave in puppies during experimental jaundice.

3. F. Schellong and E. Schütz (Zeitschr. f. d. gesammt. exper. Med., 1928, lxi, 285) found that the ends of the monophasic action current and the absolute refractory phase coincided, both in normal and premature beats obtained from frog's ventricular strips. The duration of the relative refractory phase was the same in both types of beats. Subsequently, Schütz (Zeitschr. f. Biol., 1928, lxxxvii, 219) observed that the synchronism of the ends of the monophasic action current and the absolute refractory phase persisted at low and high temperatures and also when the ventricle was exposed to an excess of K or Ca.

4. The reviewer has recently obtained three records, from patients with no clinical evidence of cardiac hypertrophy, in which the QRS and T deflections were large and inverted. The change was ascribed to a shift in the anatomical axis of the heart.

5. Although Messerle (Zeitschr. f. d. gesammt. exper. Med., 1928, lx, 490) also found an increase in the T wave during exercise, Benjamin (N 10) and Herxheimer (Zeitschr. f. klin. Med., 1924, xcviii, 484) under similar circumstances noted a decrease and inconstant changes in the T wave respectively.

6. H. L. Otto's findings (Journ. Pharm. Exper. Therap., 1928, xxxiii, 291) fall in line with the view that alterations of the T wave are caused by localized changes in the activity of ventricular muscle. He found that mild stimulation of the left accelerator nerve increased the height of the T wave and caused it to fuse with the R wave. Mild stimulation of the right accelerator, on the other hand, produced a decrease or inversion of the T wave. Stimulation of the left accelerator would presumably affect the left side of the heart more than the right and stimulation of the right nerve would have the opposite effect. It has been shown that the effect of accelerator nerve stimulation is an abbreviation of the contraction time of cardiac muscle (C. J. Wiggers and L. N. Katz, Amer. Journ. Physiol., 1920, liii, 49).

CHRONAXIE

Testing Excitability by Means of a Time Factor

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I. Historical and theoretical part. The excitability of a tissue is the property it possesses of answering by a change in shape, state or position to a modification occurring in the surrounding medium. That modification of the medium we call a *stimulus*. A stimulus may be either of a mechanical, thermical, luminous or electrical nature. The stimulus evokes in the tissue an excitation, and this excitation, if transmitted to the active organ, becomes perceptible either by a muscular contraction, or a glandular secretion or by a change in the electrical state of nerves, muscles or glands. The real nature of the excitation, of the modification produced by the stimulus *in the excitable tissue*, we do not know. It may be imagined, according to the work of Nernst, to be an unequal distribution of ions at the boundaries of distinct colloidal phases: a hypothetical view that lacks, however, definite experimental confirmation.

Thus, the nature of the excitation being unknown, the excitation itself is a phenomenon whose direct measure is beyond our reach. But the stimulus is easily accessible. We may choose the more suitable one and we can vary at our convenience its intensity and its active duration. As everybody knows, the electric current is the stimulus generally used in laboratories and hospitals, because it is easy to handle, because its strength is easy to graduate, and because the modifications it produces in tissues are reversible: this means that, a short time after the electric current has been stopped, the organ returns to the same conditions as before the experiment was started and is ready for a new excitation. The constant current, the induction shock and the condenser discharge are the three most usual forms of the electrical stimulus.

As the excitation is a somewhat mysterious phenomenon that we cannot measure, it is by the measurement of the just efficient stimulus

[1] I am very grateful to Prof. L. Lapicque who was kind enough to look over this paper and who suggested to me some corrections and valuable additions to it.

that the excitability of a tissue can be tested: one must search to find the smallest stimulus capable of producing the characteristic reaction.

For a long time the regular procedure in order to determine the level of nervous or muscular excitability has been to search for this threshold only. Weak currents of progressive intensity were used, and the stimulated organ was closely watched in order to detect the moment of the first response to the electric current, i.e., a light contraction, in case a muscle or a motor nerve were stimulated. It is customary to say, when the threshold intensity is weak, or when the threshold is high, or, in other words, when a rather strong current is necessary to reach that threshold, that the excitability is weak. Consequently the threshold method, for a long time the only one in use, defines the excitability by one single characteristic of the stimulating current, by its *intensity*. Neither the nature of the current, whether a constant one, an induction shock or a condenser discharge, nor the duration of its action, were taken into account in experimental work.

How could such a procedure be justified? How could it for so long a time be accepted in our laboratories?

A brief survey of the work of one of the pioneers of electrophysiology, Du Bois-Reymond, will give us the answer to these questions.

Du Bois-Reymond[2] established the laws of excitability which he considered to be fundamental, by experimenting upon that standard object which has been called the physiological rheoscope, the nerve-muscle preparation of the frog. The muscle used was the gastrocnemius, the nerve used was the sciatic.

If a constant current be applied to such a preparation, contraction will appear only when the current is made or broken. No appreciable modification will be noticed during the passage of the current. Du Bois-Reymond concluded that the agent which gives rise to the excitation is not the electric current in itself, but the variations in intensity of that current.

Other observations seemed to confirm this conclusion. Suppose we send an electric current through a muscle. The result will be a contraction when the current is made at a certain level, X. Then the muscle relaxes while the current continues to flow. A second contraction will take place if the current becomes 2 X. Similary we will observe two isolated contractions for each diminution of the current intensity from 2 X to X and from X to O. Such a stimulus that increases or decreases

[2] For the bibliography of older papers, see Lapicque's book (9).

in a stairway will produce a muscular response for each step, provided that the differences of intensity are sufficient.

But, according to Du Bois-Reymond, the speed of making or breaking the exciting current also plays a rôle. If the current passes suddenly from the level O to the threshold X, the muscle contracts, but the muscle remains at rest if the intensity of the current is increased gradually.

These facts may be expressed in the following words: to be efficient, the making slope of the current must be steep. But great differences are to be observed between the different kinds of muscular tissue. The plain muscles admit of a very much slower making slope than the striated ones. If we conclude with Du Bois-Reymond that the exciting agent is only the variation of current intensity, we must accept the following natural consequences of his law: a constant current, if steady, has no efficacy at all; it is not stimulating and consequently the duration of its passage is immaterial. Only the variation in the intensity of the current must be taken into account.

As a matter of fact, Du Bois-Reymond had investigated whether the level of the threshold for the making of a current is displaced by variations in the duration of the passage of that current. In the sciatic-gastrocnemius preparation of the frog, he did not find any differences in the threshold if the current was applied for a second or for half a hundredth of a second. Thus, the level of the threshold seemed to him to be independent of the length of action of the stimulus. The intensity alone of a steep stimulus plays a rôle in the determination of the degree of excitability.

For more than 60 years, lecturers on general muscle physiology taught this fundamental error, although some facts should have induced them not to accept Du Bois-Reymond's law without further inquiry.

Thus, for instance, Fick had seen, as early as 1863, that the stimulating current, applied to the muscles of Anodonta (the river mussel), becomes inefficient if applied for too short a time. He found on the other hand that the limit slope of establishment, the slope beyond which the stimulus is without action, spreads wider than in the frog. Generalising, he threw doubts on the validity of Du Bois-Reymond's law even for the frog's muscle. He thought that even in this latter object the duration of the stimulating current must have some importance. This factor escaped Du Bois-Reymond's attention because he worked on the frog, where the periods to be considered are much shorter than in Anodonta, whose muscular contractions are much slower and much more leisurely than those of the first-named animal.

In 1867, Brücke showed that a curarized frog's leg is still excitable by long currents, after the very short currents have become inefficient.

In 1870 Engelmann found on the rabbit's ureter that the threshold rises when the length of action of the current shortens. His methods of measuring time were, however, very crude. The more or less prolonged contacts were produced simply by hand, following the beats of a metronome. And yet the variation of the threshold's intensity with the duration of its action appeared clearly. Here are Engelmann's figures:

Threshold of the excitability of the ureter of rabbit (Engelmann, 1870)

INTENSITIES OF CURRENT (CM. OF RHEOCORD)	DURATIONS OF CURRENT (SECONDS)
4000	0.25
500	0.5
50	1
25	2
15	3
12	4
11	5
10.5	6

Engelmann has also made observations on the microscopic fauna of stagnant water. A drop of water containing some animalcules is put on an object holder fitted with two electrodes. A constant current is sent through, and one can see that the different animals react differently if the current is a long or a short one. There is a striking relationship between the normal speed of the animals and their reaction to the electric current. The shortest currents induce a contraction in striated muscles of small Arthropoda like Cyclops (the key being managed by hand), but a high intensity is necessary to reach the threshold if the current is short.

Some Infusoria, like *Stylonichia mytilus, Oxytrichia pellionella, Halteria grandinella,* whose movements are quick, are also stimulated by rather short currents. *Chilodon,* and *Amphileptus* call for longer durations. The duration must reach one second or more in the case of *Amoeba,* which proceeds only slowly with a creeping movement.

These findings of Fick, Brücke and Engelmann should have called the physiologists' attention to the importance of the time factor in the excitation process and should have led them to examine whether the excitability of the frog's muscle is qualitatively different from that of

Anodonta, or from that of the plain muscles in the ureter. But so great was the authority of Du Bois-Reymond that his erroneous conclusions have been taught for half a century in all the physiological laboratories: the duration of the stimulating current was said not to have any importance in the determination of the threshold of excitability. Only the *intensity* and the *quickness of variation* must be taken in consideration. One must add however that the error was a natural one, since the object used was nearly always the frog's muscle which is hardly suitable for such investigations and that the nerves and muscles of man and mammals demand even shorter times. To discover the laws of muscular excitation, a more delicate method of experimentation, a more precise technique, and more suitable objects upon which to apply them, were required.

In 1892, the Dutch physicist Hoorweg showed the inadequacy of Du Bois-Reymond's law. His experiments were carried out on the muscle of man. Using condenser discharges, he searched for the threshold of the minimal contraction, determined the corresponding voltage, and tried to find out in what measure this threshold voltage varied with the capacity of the condenser.

In such an apparatus, the formula of the discharge is an exponential one, i.e. a rather complicated one to calculate. Furthermore, it does not admit of a strictly limited duration of the passage of the current. But it is sufficiently accurate in practice to say that the discharge current takes (in order to fall to a given fraction of its initial intensity) a time which is proportionate to the capacity. Consequently, if we keep the resistance invariable, the duration of the active discharge will vary as the capacity. The result of Hoorweg's investigations were the following: when the threshold capacity decreases, the threshold voltage V increases. The quantity (C V) decreases regularly and the energy ($E = 0.5CV^2$) shows a minimum. This is expressed by the diagram in figure 1.

Hoorweg considers the curve of voltages to be an equilateral hyperbola. The curves of the quantity of electricity and of the energy are drawn from the formula of an equilateral hyperbola. This formula, as we shall see, does not exactly fit the experimental data, and consequently has had to be abandoned. Nevertheless it represents an important stage in the history of the question.

Georges Weiss, in particular, studied this problem in 1901. He made on motor nerves of the frog experiments similar to those of Hoorweg. But he did not use the condenser discharge, because the shape of the

curve of the discharge is such that it is impossible to know exactly when the discharge is finished, or at least when it ceases to be efficient as a stimulus. He used the constant make current. In order to break the current after the proper duration, Weiss invented a so-called balistical rheotome, a very ingenious instrument indeed, similar to the apparatus used in artillery yards to calculate the speed of projectiles. A rifle bullet shot with a known velocity cuts a first wire, *a*, placed so as to form a short circuit in the excitation circuit. The cutting of *a* makes the current. Then the bullet meets a second wire *b* and cuts it

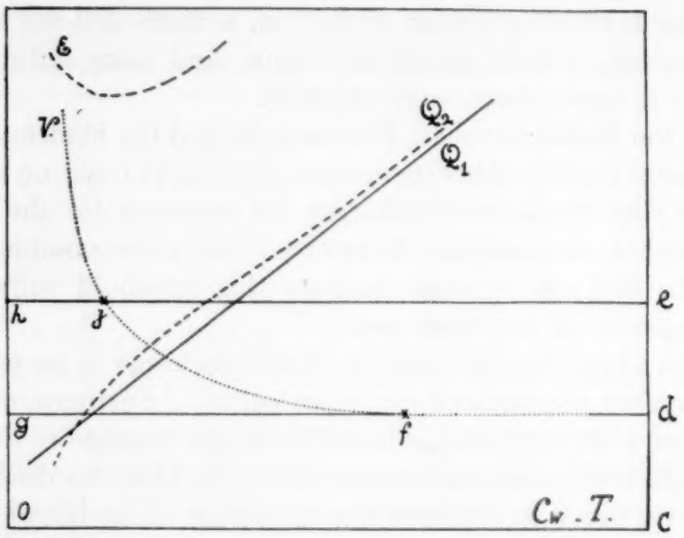

Fig. 1. *V*. Threshold voltage as a function of the capacity *Cw* (condenser, Hoorweg) or of the time *T* (constant current, Weiss, Lapicque). *Q1*. Quantity as a function of the capacity (Hoorweg) or of the time (Weiss). *Q2*. Quantity as a function of the time (Lapicque). *E*. Energy (Hoorweg). *cd*. Rhéobase. *cde*. the same, doubled. *gf*. "temps utile" (Lapicque). *hj*. Chronaxie.

in turn; *b* forms a part of the stimulating circuit. When *b* is cut, the current is broken. If the wires are close together, the current will have passed through the nerve for a short time. The velocity of the bullet is 136 meters a second. If the distance between the wires be 13 cm., the duration of the current will be one-thousandth of a second.

The figures Weiss gathered from his investigations on the frog's motor nerve allow one to construct (as a function of the duration of the current) a curve of the quantity of electricity. The quantity is obtained by multiplying V by T. The curve looks like a straight line whose origin is a positive value on the axis of ordinates. That is to say, to a given

constant quantity we must add another quantity which increases indefinitely with the duration of the current. One recognizes Hoorweg's straight line of quantities. Consequently, the curve expressing voltage as a function of time would be, as was the case in Hoorweg's work, a branch of equilateral hyperbola. Such a curve, descending from high values for the very short durations, comes nearer and nearer to a parallel to the axis of the time. This means in plain language that for a long duration of current the threshold intensity remains always at the same level. The stimulating current may be lengthened or shortened within wide limits and the threshold does not change. Thus the right half of the curve *is* in agreement with Du Bois-Reymond's law. But if we reduce the time the law fails. The intensity curve rises to the left. To reach the threshold with short currents, one must increase the intensity. And now we see the reason of Du Bois-Reymond's mistake. To find the threshold depending upon the time, the German physiologist did not try current shorter than 0.005 second. This was too long for a quickly reacting organ like the frog's muscle. Had he used, as did Hoorweg and Weiss, very precise instruments that allow of very short durations, he would have seen the curve rise to the left in an hyperbolic shape.

If it be true that for long times the duration of the stimulus has no importance, then we must conclude that only the first portions of the current are stimulating, the longer durations adding nothing to the already produced excitation. There is consequently a certain duration after which the action of the stimulus ceases. This duration has been called by Lapicque "temps utile," an expression we could translate into English by "profitable or serviceable time" or "effective period." It is the shortest duration for the lowest stimulus. So we must abandon Du Bois-Reymond's conception, that ascribed the excitation not to the current itself, but to the variation of the current, or better to the derivate of that variation. We know now that in the case of a constant make current, it is the current itself that stimulates but not the whole current, only its beginning. It stimulates even if the threshold potential is reached within so short a time that it is immeasurable, if one makes use of "right angled" waves in which the rise is shown to be perpendicular to the time axis. The roughly hyperbolic form of the curve of threshold intensities as a function of threshold times has been carefully determined by Lapicque for very different objects: striated muscle, heart muscle, plain muscle, snail's foot, filaments of Spirogyra, etc. The shape is always the same, no

matter what the excitable object may be.[3] This has been found by other experimenters as well.[4] But to attain a result, one must choose for each object a particular and different unit of time: one ten-thousandth of a second for the gastrocnemius of the frog; one-thousandth of a second for the extrasystoles of the frog's or the turtle's heart; one-hundredth of a second for the muscle of the snail's foot; one second for the frog's stomach; ten seconds for the retraction of the filaments of Spirogyra (134).

As Lapicque has expressed it, *every organ reckons the time with its proper unit.* And so it appears that there is a distinction to be made between quick and slow organs, a distinction which will justify our attempt to characterize the excitability by a time value. This latter point will, I trust, become clearer as we proceed in this paper.

That every organ should have its particular unit of time is not at all surprising: do we not use different time standards in the different circumstances of life? A member of an orchestra is late if he begins his part only a few hundredths of a second after his time. For a belated train, we count time in minutes; for a belated steamer, in hours or days. A different unit is used to express the time in which the same distance, say a mile, is covered by an airplane, or a pedestrian or a snail, i.e., seconds, minutes, hours or days. In such cases, we may determine very accurately this characteristic time; can we make such a measurement accurate enough for each kind of excitable tissue? This measure is in fact given by the *Chronaxie.*

We have to thank Lapicque, first for calling our attention again to this "relative cellular time," a glimpse only of which had been caught half a century ago; and secondly, for proposing a simple and practical method for applying this measure, the results of which by this time are numerous, and which has opened up a large field for new researches, as will be shown in the present review.

Since the curve which shows the relation of the threshold intensity to the duration of the stimulating current is always of the same shape (the unit of time only being different for different tissues) Lapicque at

[3] Monnier (167) was able to measure the time factor of the excitability in Lillie's steel wire model (artificial nerve).

[4] Everybody knows that, according to Keith Lucas, the time-intensity curve of excitability shows a much more complicated shape. In the sartorius of the frog, Keith Lucas (105) believed he could distinguish three different degrees of excitability α, β and γ giving rise to three different curves. Lapicque, in his book (9, p. 249) has made a thorough criticism of Keith Lucas' view point. The latter has been recently supported by E. Th. Brücke (44).

first tried to find an algebraical formula which would fit all cases. Such an expression, in order to represent the facts exactly, must include what is called by the physicists a *constant of time*. This constant, deduced from a few experimental figures should give the time characteristic we are trying to find.

The hyperbolic formula of Hoorweg and Weiss seemed at first to solve the problem very simply. For several stimuli of different durations t, this formula undertakes to express as follows the threshold intensity i.

$$i = a / t + b$$

a and b are two constants which can be algebraically calculated (system of two equations with two unknown factors) by means of two measurements of i corresponding to two different values of t. Weiss had chosen as a characteristic the ratio a/b of the constants, but he did not ascribe to this ratio a definite meaning. Lapicque pointed out that, for the formula to be consistent, a must be a quantity of electricity, and b an intensity. (b is the threshold intensity for infinite durations = cd in fig. 1 = rheobase). It follows that the ratio a/b will express a *time*, a duration. It happens that this characteristic time can be found as follows:

$$i = \frac{ab}{bt} + b$$

$$i = b\left(\frac{a}{bt} + 1\right)$$

a/b being a value of time, let us indicate the ratio by the Greek letter τ.

Then

$$i = b\left(\frac{\tau}{t} + 1\right)$$

τ would be the constant of time looked for. Let us observe that when t increases, $\frac{\tau}{t}$ diminishes and approaches zero. For a long stimulus, i approaches the constant value b. If $t = 10\tau$ then $i = 1{,}1b$. Likewise, in the particular case where $t = \tau$, we get $i = 2b$. The constant of time (chronaxie) would be the duration of stimulus corresponding to the threshold intensity twice as great as the threshold found for infinite durations (see fig. 1). But, since the beginning of his researches,

Lapicque has observed that it often happens that the hyperbolic formula is not in agreement with the series of experimental figures. It follows, as shown in Lapicque's book (pp. 204 seq.) that in a given experiment, calculations by means of this formula may lead, according to the chosen durations, to very different values of the constant of time. Recently Lapicque proved that Weiss and Lapicque himself had been wrong in measuring the constant of time in the turtle. He explains this error by the lack of exactness of the hyperbolic formula used (138). Lapicque introduced into the formula empirically a correction; then, starting from Nernst's hypothesis, he searched a great while for a physicochemical theory of the electrical excitation which would admit of a satisfactory formula. Since he realized that the phenomenon was a very complex one and that every adequate expression would be difficult[5] to calculate he confined himself to a purely experimental measurement.

In order to define an excitability, we have to know two characteristic values, two *parameters* non-reducible one to the other: a parameter of intensity and a parameter of time. This had been shown by all theoretical researches and by all the approximate formulae. Any threshold may be influenced by both parameters; but we eliminate the influence of time in searching for the threshold with a duration long

[5] The curve of quantities is not a straight line; it is composed of three parts:

1. For short times, a curve concave towards the axis of times, and rising as the square root of the time (Nernst's law).

2. For long times, a straight line passing through the origin, i.e. increasing proportionally to the time.

3. Between these two parts a connecting arch, bent in a direction contrary to that of the first part.

Thus the curve of intensities cannot be a hyperbola. It is expressed as nearly as possible by the following formula

$$i = a \frac{\sqrt{t + \theta + \sqrt{(t - \theta)^2 + 0.16\,\theta^2}}}{2t}$$

in which t = duration of stimuli (constant current).

a = threshold of intensity for long durations.

θ = the constant of time sought for.

Every physiologist will agree with Lapicque in admitting that this formula found by him in 1924, must not be substituted for the empirical definition of Chronaxie as given by him fifteen years earlier. Nevertheless, if, in a special case, an exact interpolation formula should be needed, it would be possible to pass from the chronaxie τ to the constant θ by means of the simple equation

$$\theta = 3.8\tau$$

(see Lapicque's book, 9, pp. 212 seq.).

enough. That threshold, independent of the duration, has been called by Lapicque *rheobase*, i.e., the base of the currents (*b* in the calculations above; *cd* in fig. 1). Above that base, the threshold rises when the duration is made short enough. The threshold becomes continually higher as the duration becomes shorter, according to the curve we know.

In order to know the comparative influence of time, it suffices, since the curves have always the same shape, to determine the duration necessary for an increased threshold value, which we shall always choose so that it bears a certain definite ratio to the rheobase: Lapicque has chosen the ratio 2/1.

The duration for which the threshold is reached with an intensity equal to twice the rheobase is thus the *time characteristic*, the so called *chronaxie*[6] which has been adopted by nearly every physiologist. Lapicque insists upon the *experimental* definition of chronaxie which is independent of the uncertainties and changes in the theory. Furthermore, the chronaxie, defined in this way, must be proportional to the *constant of time* of any mathematical formula which would translate accurately enough the physiological facts. Research workers will be pleased to know that they can obtain it without any mathematics.

We need two direct measures only: the first one is the rheobase, i.e., a threshold of intensity which we reach by experiment, but with sufficiently long durations of current; then, with twice this threshold intensity, we have, by an analogous experimental method, to find the corresponding *threshold of duration*. This is the chronaxie.

Any other point of the curve could also be used by its coördinates for defining the excitability. Why then should we choose the chronaxie in particular? Is there no other point to be preferred? At first sight, there is another point that seems better. This point is located where the curve ceases to show an upward directed concavity and continues into the straight line whose ordinate is *b*, that part of the curve that is parallel to the axis of time.

This point corresponds to the shortest time for the weakest active intensity. It indicates what Lapicque has called *temps utile*.

[6] The *chronaxie* is sometimes translated into German by *Kennzeit*. The *rheobase* is called in German *Grundwert* (Hansen and Schroeder, 103). Nevertheless, the terms *chronaxie*, *rheobase* are used, even in German (Ebbecke, 5, 6; Field and Brücke, 83, etc.) and it appears to be very convenient, because of their greek, i. e. international character, to keep to the original terms of Lapicque, who proposed the notion.

This table shows that the *temps utile* diminishes as we pass from slow to quick muscles.

The so called Lapicque's "Temps utile" (serviceable time or effective period)

	second
Aplysia's muscle	0.80
Crab: Claw muscle	0.30
Turtle: heart	0.08
Snail: foot	0.05
Frog: gastrocnemius	0.003

[L. and M. Lapicque (123).]

At first sight, it would seem very advantageous to pick out the *temps utile*. But let us consider the curve. We see that on the right, it gets nearer and nearer to the time axis. Consequently, it is not possible to note the exact point where the curve becomes parallel to that axis. Moreover, we must realize that in this part of the curve, for a very small variation of intensity, the variation of the threshold duration may be considerable. This makes a correct experimental measurement difficult. Thus it is in the left part of the curve that we will endeavour to find the characteristic duration. And if we are satisfied with an empirical definition of the chronaxie, we shall choose the simplest method: we must take an intensity value very much larger than b. Let us take the double of b and with this value $2b$, let us look for the threshold duration of the stimulating current.

Such is the method generally used in practice.

II. Technical procedure.[7] The experimental measurement of the chronaxic times can be made either by the condenser discharge or by the flow of a constant current.[8]

1. In the first case, the experiment must be so arranged that it is possible to vary, first the loading potential, and second the capacity used, for the duration of a discharge varies with the capacity. One must use a battery, fitted with a rheotome or potentiometer. A subdivided condenser giving the capacities wanted, a key with two directions, and, in the discharge circuit a strong resistance disposed in series, finally another resistance shunting the circuit (108a, 108b, 109, 110).

It is well known that the duration of the condenser discharge is

[7] For more details, see papers issued by Lapicque and his school.

[8] Wertheim Salomonson invented a method according to which chronaxie can be measured by means of induction shocks (199, 201).

proportional to the capacity used, as long as the resistance R of the discharge circuit does not vary. It is very likely that the object in practice would show variations in resistance. To make these variations immaterial, we shall introduce in the circuit a strong resistance, from 15,000 to 20,000 ohms.

Now let us see what the practical procedure is.

A great capacity is to be taken, a capacity that is practically infinite, two microfarads for instance if one experiments with the frog's muscle, and one searches for the threshold with this capacity whose discharge time is very long. One notes the voltage corresponding to the smallest perceptible effect, say, the weakest muscular contraction. This voltage is doubled, and then, one searches for the lowest capacity Cw, still giving the same reaction. Lapicque pointed out that the chronaxie is proportional to the product of the capacity Cw and the resistance of the discharge circuit:

$$\text{Chronaxie} = \tau = K\,R\,Cw.$$

We shall in a moment determine the value of K.

2. If, on the other hand, we use a constant current, the technical equipment will be slightly different.

We shall need: a battery, a potentiometer for varying the voltage, a rheotome, acting as a key, for varying the duration; and a system of peripheral resistances.

The method of procedure is the following:

One searches for the rheobase by closing repeatedly the circuit with increasing intensities, the current being each time allowed to pass for a rather long period, 0.5 to 1 second in the case of the frog's muscle. When the threshold is reached, the threshold voltage is doubled and by means of the rheotome, the shortest effective duration is determined. This is the chronaxie. The most delicate part of this equipment is the rheotome, for it is necessary to get very short but also very exactly measured durations.

A rifle, as used by Weiss, is not very practical.

Several other apparatuses have been constructed, in which two keys make and break the current: such are the pendulum of Keith Lucas, (105) the ergésimètre of Strohl, (189) the chronaximeters of Lapicque (9, p. 311) and of Noyons (170).

Each of these apparatuses can be used to advantage in different experiments. In all of them, the distance between the keys of make and break can be changed, in order to vary the duration of the current.

3. The use of a constant current leads to a value of the chronaxie that is directly expressed in fractions of a second. If condensers are used, the figures correspond to fractions of microfarads. This result can be considered as sufficient, as the capacity is always proportional to the discharge time, if the resistance does not vary. But if the chronaxie is to be calculated in seconds, the well known formula is applied: $T = CwRK$.

Cw and R can easily be measured. As to K, numerous experiments made under Lapicque's direction (127) have led to a value $K = 0.37$. The formula, in order to get τ in seconds is then: $\tau = Cw$(Farads) by R (Ohms) by 0.37.[9]

The experiments have shown that the results obtained by operating either with the constant current or with the condenser agree satisfactorily.[10]

4. Several other characteristic threshold durations, different from the chronaxie, have been elaborated. For instance, attempt has been made to relate chronaxie and energy. In the condenser method, Hoorweg and Weiss admitted that the threshold energy passes through a minimum (see fig. 1). And Lapicque in his first papers thought he had experimentally established that the chronaxie was equal to the duration corresponding to the minimal expense of energy. The curve obtained from experiments on the frog shows that this minimal energy corresponds to a time equal to the chronaxie. But later, working with constant currents on the slow muscles of the Crab or of Aplysia, Lapicque came to the conclusion that the relation between chronaxie and energy does not always exist. In these slow muscles, no minimal energy is to be found. On the contrary, the curve of energy in function of time may show a maximum. Consequently we must give up such characteristics of excitability as the one proposed by Waller in 1899, the so-called Waller's characteristic. In practice, this value proved itself variable in a ratio ranging from 1 to 250 according to the experimental conditions.

[9] The calculations of Bouman (21) and Monnier (168) have recently brought a mathematical confirmation and signification of this experimental value. ($K = \frac{3}{8} = 0.375$). According to Vogel (197) the value of K would be 0.51.

[10] For lack of space, the question of the relations of the chronaxie to short interruptions which cut the stimulating current cannot be discussed in this review. See the publications of Lapicque, Laugier, R. Dériaud, Ozorio de Almeida, A and B. Chauchard, and H. Piéron (74, 75, 76, 142, 158, 173, 175). As a general rule, it appears that a lacuna interrupting the stimulation current has an economical result as regards the total amount of energy expended in order to reach the threshold.

It seems also safer at present to rely upon the logarithm of θ, as proposed by Hill and Keith Lucas, because this logarithm of θ is based on complicated calculations, which themselves imply that we already possess a satisfying theory of the excitation in general. These questions are extensively discussed in the book of Lapicque (9, p. 66 and p. 162 seq.).

It is best to keep to an empirical number, like the chronaxie, without attempting at present to penetrate more deeply into the intimacy of the observed phenomena.[11]

We can by the chronaxie measure the quickness of excitability, as we can measure the temperature with the thermometer, even without knowing the mathematical theory of the instrument.

III. JUSTIFICATION OF CHRONAXIMETRIC METHODS. We must justify the change which has been imposed upon the habits and minds of the experimenters, who have now to consider *time* where they used to consider *intensity*.

Was that change really necessary? The answer to that question is the object of the following pages.

A. *The point of view of experimental practice.* All physiologists know how uncertain is the determination of a threshold intensity. Its level is influenced by a great number of external conditions that have nothing to do with the biological properties of the examined organ.

The electrical resistance is a very important factor in the determination of the threshold voltage. The resistance may not vary very much within the duration of one single experiment, but it may be entirely different for another animal.

It is consequently impossible to compare the threshold excitability of a muscle in a frog with that of another frog, while a comparison between the excitability of a frog's muscle with a toad's or a turtle's muscle is entirely out of the question. On the contrary, we shall see that such a comparison is possible and leads to fruitful conclusions, if the chronaximetric standard has been chosen.

The position, shape, size and grade of imbibition of the electrodes play a rôle in the level of the galvanic threshold. It results from these conditions that the values found for the threshold intensity cannot be compared if the place of the electrodes has been changed.

In spite of the opinion expressed by some authors,[12] the same incon-

[11] Cf. also the "linear constant" of Ph. Fabre (81, 82) and the formula recently proposed by H. Lasalle (159, 160).

[12] Watts (198), Adrian (13, 14).

venience does not exist for the chronaxic method. The rheobase may vary with some experimental conditions of the research, the chronaxie varies only with physiological conditions, for instance, with the temperature.

Here are some figures that are in this respect very enlightening:

STIMULUS	RHEOBASE	CHRONAXIE
	milliamp.	*sec.*
Condenser discharge	4,4	0,00008
Induction shock	6,1	0,00008
Constant current	2,3	0,00008

[*Wertheim Salomonson* (200).]

B. *The purely physiological viewpoint.* We shall see that the notion of chronaxie explains and coördinates a number of scattered points which have not appeared so far to have any relation to each other.

1. Muscles. a. It is well known that the quickness of reaction is quite different in striated and smooth muscle. The latency, the period of contraction, and the period of relaxation vary in length according to the function of the muscles. The jump of a frog is quicker than the jump of a toad. The movements of the tail in lobsters, shrimps and crayfishes are quicker than those of the claws. In the rabbit, the red muscles contract more slowly than the white ones. Smooth muscles are the slowest.

ANIMAL	MUSCLE	CHRONAXIE σ	LATENCY OF CONTRACTION σ	DURATION OF CONTRACTION
				sec.
Rana	Gastrocnemius	0.3	10	0.1 (jump, swim)
Calyptocephalus	Gastrocnemius	0.8	22–23	0.45
Bufo	Gastrocnemius	0.9		Slower than Rana
Turtle	Extensors and flexors of leg	1–2		1.0
Crab	Claw	11–14		0.5
Frog	Stomach	100		15–20 (mix the food)

[L. and M. Lapicque, T. Muhm (114b, 121, 122, 123, 126, 129, 138, 140).]

The old method of measuring the excitability by determining the threshold does not lead to any explanation of these differences. On the contrary, the comparison of the chronaxies gives an account of the

different functional reactions. For the slow muscles, long chronaxies; for the quick muscles, short chronaxies.

b. For a long time, a difference of quality was supposed to exist between the excitability of smooth and striated muscle.

Induction shocks are excellent stimuli for striated muscles. They do not stimulate the smooth ones. Constant current on the contrary is efficient in both cases. The measure of the chronaxie will explain these differences.

The induction break shocks are very short, they last about one-thousandth of a second. And the smooth muscles are excited only by long currents, because their quickness of excitability is small, because their chronaxie is long. Induction shocks are too short stimuli for the excitability of smooth muscles. On the contrary, striated muscles have a great quickness of excitability and react perfectly to these short currents. In the palatal muscle of the tench, two different chronaxies can be found: 0.26 and 33 σ. The first one corresponds to the contraction of striated muscles. The last one measures the excitability of a particular kind of muscle, intermediate between striated and smooth muscles (Bouman, 20). It means that the use of chronaximetric measures enables us to distinguish the different kinds of fibres present in a muscular system.

c. The study of the action of cold on muscular excitability is another example of an interesting relationship. Cold was formerly supposed to decrease the faradic and to increase the galvanic excitability. As a matter of fact, cold lengthens the chronaxie of nerve and muscle (L. and M. Lapicque, 122, 123, 125, 139; G. Filon, 84; Van der Heyde, 192; Bourguignon, 1). Cooled muscles become less influenced by short currents as induction shocks. Cold not only lengthens the muscular chronaxie; it lengthens and lowers the contraction and diminishes the speed of the motor nervous impulse. Later on, we shall study anew the significance of this interesting relationship.

Fatigue increases the chronaxie two or three times. It lengthens also the myographic diagrams. Adrenalin dispels the effects of fatigue and also shortens the chronaxie of the fatigued muscle (M. Lapicque and M. Nattan-Larrier, 147).

Stretching striated cardiac or smooth muscles diminishes their chronaxie (Henri Fredericq and Marcel Florkin, 91, 97, 98). Chronaxie of smooth muscles has its minimal value in isotonic solutions.[13] The time factor is lengthened in hyper- as well as in hypo-tonic liquids (Florkin, 88).

[13] The effect of anisotony on the chronaxie of striated muscle has been studied by L. and M. Lapicque (133), H. Renauld (176) and H. Laugier (156, 157).

d. Chronaxie throws a new light on the problem of the differences noted in regard to the quickness in making the current. A striated muscle contracts only when the change in strength is carried out abruptly. A smooth muscle will still contract when the circuit is closed much more slowly.

According to Lapicque, the rheobasic current is efficient only when its making does last less than ten times the chronaxie.

The make of a current lasting 10 seconds stimulates the muscle of Anodonta; that of a current lasting 1 second stimulates the stomach of frog, and that of a current lasting 0.001 second stimulates the gastrocnemius of frog.

2. Heart. The chronaxie of the heart can be measured as well as the chronaxie of other muscles. We test it by producing extrasystoles and searching for both their time and duration thresholds.

The heart muscle is a very convenient object for demonstration and teaching. For, in conformity with the "all or nothing" law, the threshold can be easily found. The contraction is each time maximal and easily noticeable.

On the contrary, the so-called perceptible minimum of a gastrocnemius is often a very small contraction, very difficult to perceive.

In the heart, the chronaxie of the sinus and of the auricles is always the same as the chronaxie of the ventricles. (Mmes M. Lapicque and Cath. Veil, 151).

The actual myocardium has thus only one and the same quickness of excitability all over the heart. But if the exciting electrode is applied to the heart bundle (or funnel), which now assumes the rôle of a conductor of the impulse from one cavity to the other, the chronaxie appears to be three times longer. This decrease of excitability in the auriculo-ventricular track is, in my opinion, caused by the normal diminution of the excitation wave in that same track.

Heart's chronaxie

SPECIES	a VENTRICLE	b BUNDLE OR FUNNEL	RATIO a:b	AUTHOR
Dog	2 σ	6 σ	1:3	Mme. Lapicque and Henri Fredericq (146).
Frog	3 σ	9 σ	1:3	M. Lapicque and C. Veil 151, 152, 193
	0.7 M.F.	2.2 M.F.	1:3	
Turtle	9 σ	29σ	1:3	
Fish	0.7 M.F.	2 M.F.	1:3	

In the Purkinje tissue of sheep and dog, Mmes M. Lapicque and Cath. Veil found the same long chronaxie as in the His' bundle (152). Junctional tissues that have a long chronaxie also conduct most slowly the contraction wave. The long chronaxie of the His' bundle is responsible for the a-c interval of the jugular pulse, for the P-R interval of the electrocardiogram. I know that when I propose this latter explanation, I differ from Lewis, Hering and other cardiologists, who believe the speed to be greater in the His bundle than in the auricular or ventricular myocardium. They explain the interval a-c or P-R by the presence of Tawara's node on the path followed by the excitation. But the measurements of the chronaxie in junctional tissues throw doubt on that interpretation. Provisionally I believe that to a long chronaxie, i.e., to slowness of excitability, corresponds a low velocity of conduction. This interpretation is supported by the facts recently established by Mmes M. Lapicque and Cath. Veil: In the branches of the His bundle of the dog the speed of conduction is about three times longer than in the auricular tissue (155).

The ratio 3/1 that appears to exist between the chronaxies of the junctional tissue and the walls of the cardiac cavities is necessary for the passage of the excitation from the auricles to the ventricles. In fact, it has been demonstrated that poisons which modify that ratio produce allorythmy, that is, auriculo-ventricular dissociation (C. Veil, 193).

3. Action of heart nerves on heart excitability. The study of the action of heart nerves on the excitability of cardiac muscle, the action Engelmann called bathmotropic, became also fruitful when made by the chronaximetric methods.

Most textbooks call the bathmotropic action of the vagus a negative one. This means that faradization of the vagus decreases the heart's excitability, or, in other words, causes the threshold level of that excitability to rise.

As to the bathmotropic action of the sympathetic accelerator nerves, it is not mentioned anywhere.

Now, let us leave aside the textbooks and consult the original papers: then, we get the results collected in the next table.

The results are quite incoherent.

The former threshold method does not give a general rule fitting all cases. It does not show if the bathmotropic action of the vagus is positive or negative. It does not even show if there is such an action.

Bathmotropic action of the Vagus. Summary of the literature (threshold method)

OBJECT	ACTION OF VAGUS ON EXCITABILITY	AUTHOR
Eel: sinus and auricle	Unexcitable	MacWilliam
Newt: heart	Unexcitable	MacWilliam
Turtle, Frog: auricles	Unexcitable	Schiff, Bidder
Snake: auricle	Unexcitable	Mills
Menobranchus: Ventricle	Unexcitable	MacWilliam
Frog: auricles	Excitability increased	Hofmann
Turtle: ventricle	Excitability unchanged	Kronecker
Eel: ventricle	Excitability unchanged	MacWilliam
Frog: heart	Excitability increased or decreased	Engelmann
Dog: ventricles	Excitability increased	Schiff
Dog: ventricles	Excitability unchanged	MacWilliam
Dog: auricles	Excitability decreased	MacWilliam
Dog: ventricles (fibrillation)	Excitability decreased	Bayliss and Starling

But, if we measure the quickness of excitability rather than its level, we get the following figures:

Chronaxie of the ventricles

	A. REST OF THE VAGUS	B. FARADIZATION OF THE VAGUS
	σ	σ
Dog	1	0.5
	1.5	0.75
	2	1
	3	0.9
Frog	3.5	2.5-2
	5	2.5

[Henri Fredericq (89, 90). Mme Lapicque and Mlle Veil (153). Field and Brücke (83).]

Similar results have been found in turtle and fish. Mmes Lapicque and Veil showed that the vagus faradization reduces in the same manner the chronaxie of the junctional tissue (a-v funnel). Thus, faradization of the vagus acts upon the heart in decreasing the chronaxie, or increasing the quickness of excitability. At first, this result seemed to me rather astonishing. Rather, it was expected to find in the experiments a lengthening of the chronaxie. But the conclusions have been confirmed in several laboratories; this fact will perhaps some time in the future be used for building up a theory of the heart inhibition by the vagus.

The accelerators are directly antagonistic to the moderators: they lengthen the chronaxie, as shown below:

Dog: chronaxie of right ventricle

REST OF ACCELERATORS	FARADIZATION OF ANSA VIEUSSENII
σ	σ
1	4.5
3.5	5

[Henri Fredericq (92).]

Thus, the chronaxic method has allowed us to dispel the apparent discrepancy between a number of facts revealed by the threshold method.

Generally speaking, drugs that moderate the cardiac rhythm act like the vagus on cardiac excitability: they shorten the chronaxie (95).

Frog: isolated heart in Ringer

DRUG	ACTION ON FREQUENCY OF HEART BEATS	ACTION ON HEART CHRONAXIE	AUTHORS
Cholin	Moderation	Shortening	H. Fredericq
Acetylcholin	Moderation	Shortening	H. Fredericq
Betain	Moderation	Shortening	H. Fredericq
Neurin	Moderation	Shortening	H. Fredericq
Arecolin	Moderation	Shortening	H. Fredericq
Pilocarpin	Moderation	Shortening	H. Fredericq
Eserin	Moderation	Shortening	Cath. Veil
Quinine and its derivatives	Moderation	Lengthening	Mme. Lapicque and H. Fredericq

On the contrary, drugs which accelerate the cardiac rhythm lengthen the cardiac chronaxie (95).

Frog: isolated heart in Ringer

DRUG	ACTION ON FREQUENCY OF HEART BEATS	ACTION ON HEART CHRONAXIE	AUTHORS
Cocain	Acceleration	Lengthening	Cath. Veil
Atropin	Acceleration	Lengthening	Cath. Veil
Strophantin	Acceleration	Lengthening	Mmes. Lapicque and Gruzewska
Digitalin	Acceleration	Lengthening	Mmes. Lapicque and Gruzewska (101)
Glyoxylic acid	Acceleration	Lengthening	H. Fredericq
Caffein	Acceleration	Lengthening	H. Fredericq
Adrenalin	Acceleration	Lengthening	H. Fredericq

There is however, no universal relationship between the frequency of rhythm and chronaxie: quinine, as we have seen, moderates the frequency but lengthens the chronaxie.

During the faradization of the vagus, the chronaxie is shortened. But that shortening is not the consequence of the chronotropic effect of the vagus, of the slackening of the rhythm. If, during the stimulation of the vagus, the heart is induced to beat at its normal rhythm by induction shocks applied to the sinus, the chronaxie keeps short (Henri Fredericq, 96; Field and Brücke, 83). On the other side, drugs which modify the chronaxie act as well on the resting apex of the frog's heart, as on the whole heart which contracts spontaneously (Henri Fredericq, 96). Thus the bathmotropic action of the vagus is a direct one, and not the indirect result of the change occurring in rate.

4. Action of the vagus on the stomach excitability. The vagus being an inhibitory nerve for the heart, but an excitatory nerve for the stomach and the intestine, one could have expected to find a lengthening of the chronaxie of the digestive tract during vagus stimulation. On the contrary, as Florkin (86) pointed out, the vagus faradization reduces the chronaxie of the frog's stomach. Thus the vagus acts always in the same manner on the excitability of its terminal organ, whatever may be its other actions (moderator or motor) on the same organ.

5. Motor nerves. The comparison of the nerve chronaxies with the other chronological factors of the functions of motor nerves leads also to very interesting results.

In the following table are compared series of nerve chronaxies with series of velocities of nerve impulses.

SPECIES, NERVE	VELOCITY OF IMPULSE	NERVE'S CHRONAXIE	
	cm. per second	*second*	
Rana: motor sciatic	3000	0.0003	L. and M. Lapicque and T. Muhm (140)
Calyptocephalus: motor sciatic	1200	0.0008	
Lombricus: nervous chain	60	0.02	M. Lapicque et C. Veil (154)
Leech: nervous chain	40	0.03	

Thus, the slowest nerves have the longest chronaxies.

Lapicque and Legendre have discovered a peculiar relationship be-

tween the diameter of nerve fibres and their chronaxie (114a, 143, 162). Their figures have been recently rectified to a certain extent by Lapicque, Desoille and Kreindler (107 and 141). Here are some figures found in frog.

MUSCLE	CHRONAXIE (σ) OF NERVE	AVERAGE DIAMETER OF NERVE FIBRES (μ)
Semi-membraneus	0.20	17.5
Triceps	0.25	16
Gastrocnemius	0.30	13
Sartorius	0.30	12
Flexor of fingers (posterior leg)	0.50	9.5

It is worth noticing that in the rabbit the nerve fibres leading to red muscles are decidedly thinner than those which lead to white muscles. And as is well known since Ranvier, the red muscles are slower than the white ones.

The thinner fibers have the longer chronaxies, and the long chronaxies correspond to low velocities of conduction. Thus, the thinner fibers are slower conductors of the impulse. In thick fibers on the contrary, the excitation progresses more quickly, as if the way were easier.[14] It is also possible to lengthen the nerve chronaxie by compressing slightly a portion of the nerve trunk (L. Lapicque and Laugier) or in swelling the myelin by means of a poison (L. Lapicque and Legendre, 113, 144) or in making the fibres become thinner, as occurs in hypertonic solutions (L. and M. Lapicque, 133).

There is another element of the nervous function that can be measured: the duration of the action current, of the negative variation. The duration is measured on a graph obtained by means of the capillary electrometer or the string galvanometer.

In 1914, Lillie demonstrated that the action current is longer in the slow conducting nerves.

It is not to be doubted that a relationship must exist between the duration of action current and chronaxie. According to Lapicque, the

[14] According to Erlanger and Gasser, in many nerves, the stimulation caused by an induction shock is followed by several excitation waves of different speed. This effect seems not to exist in the phrenic nerve whose fibres all have the same average diameter (100).

ascendent phase of the electrical variation lasts three times as long as the chronaxie.[15]

6. Relations between motor nerve and muscle. In 1906, Carlson investigated the relationship existing between the speed of nervous conduction and the abruptness of the muscular twitch.

SPECIES	MUSCLE	NERVE	DURATION OF CONTRACTION	SPEED OF NERVOUS IMPULSE
			seconds	*meters per second*
Frog	Gastrocnemius	Ischiadicus	0.10	27
Snake	Hypoglossus	Hypoglossus	0.15	14
Lobster	Claw's M.	Ambulacral	0.25	12
Crab	Claw's M.	Ambulacral	0.50	6
Limulus	Claw's M.	Ambulacral	1	3.25
Limax	Foot	Pedal	4	1.25
Pleurobranchoea	Foot	Pedal	10	0.75
Ariolimax	Foot	Pedal	20	0.40

(Carlson).

It appears that slow muscles have a long chronaxie, slowly conducting nerves also.

The value of chronaxie will consequently enable us to foretell the speed of the nervous impulse, the quickness of muscular contractions and the duration of the electrical variation of the nerve.

7. Physiological isochronisms. Curarization. But we can even go further. The systematic investigation of muscles and their motor nerves brought Lapicque to the following very striking conclusion: *a muscle and its motor nerve constitute an isochronical system.* They have the same chronaxie. Two adjacent muscles may have a different chronaxie, each of them has the same chronaxie as its motor nerve. The isochronism of the neuromuscular system is, according to Lapicque, a necessary condition for transmitting the excitation from nerve to muscle.[16] This hypothesis has been used to build up a theory of curare poisoning (9, 124, 128, 130a, 131, 132).

[15] This view seems not to be quite in agreement with some recent data of Erlanger and his coworkers (experiments with the x-ray oscillograph).

[16] If local changes of temperature are applied to some parts of the auricles of mammals, an heterochronism may appear, which seems to be responsible for the stopping of intracardiac conduction between the cold and the warm parts (F. Bremer and L. Deloyers, 36).

After the curare has been applied, the muscle is still excitable and so is the nerve. But the nervous impulse is no longer transmitted from nerve to muscle.

Curarization is thus characterized by the abolition of indirect muscular excitability and the preservation of the direct one.

Other drugs than curare can determine a similar condition.

According to Lapicque, the curarizing drugs abolish the nerve-muscle isochronism just mentioned. The ratio of both chronaxies is no more 1/1. When the action of the drug is felt, the ratio becomes 1/2 or 2/1. Curare is a drug which progressively lengthens the muscular chronaxie without modifying the nervous chronaxie. As soon as the muscular chronaxie has become twice as long as the nervous chronaxie, the indirect excitability is lost. The muscle can no more be stimulated through its nerve.

If the theory is right, we may foresee four kinds of curarization:

NUMBER	CHRONAXIE OF NERVE	CHRONAXIE OF MUSCLE	EXAMPLE
1	Unchanged	Lengthened	Curare, spartein, fatigue
2	Unchanged	Shortened	Veratrin
3	Lengthened	Unchanged	?
4	Shortened	Unchanged	Strychnin

As a matter of fact, cases like number 1, 2 and 4 have actually been discovered. The third possibility still lacks an actual demonstration.

These conclusions have been controlled by a crucial experiment. If used separately, veratrin and strychnin are curarizing drugs; if used simultaneously, they are not. Why is this? Because, when veratrin has shortened the muscular chronaxie and when curarization has taken place, owing to a heterochronism between nerve and muscle, we can restore the isochronism by shortening now the nervous chronaxie, which had not been influenced by veratrin. And what do we see? Both chronaxies, nervous and muscular, having varied in the same direction, the isochronism is reëstablished. The indirect stimulation of the muscle is henceforth possible again. Is this not the proof that isochronism is a necessary condition for the transmission of the nervous impulse to the muscle?

This theory of curarization is quite different from the classical view according to which the curarizing drugs do not influence either the excitability of muscles or the excitability of nerves, but paralyze

the end-plates (whose existence seems to me more theoretical than real).[16b]

Some more interesting examples of isochronism will be mentioned below.

M. et Mme Chauchard have shown that the chronaxie has the same value all along the motor track, from the cerebral cortex to the muscle (62).

In the dog, they found a chronaxie of 3 ten thousandths of a second in the grey substance of the brain, in the corona radiata, in the internal capsule, in the foot of the cerebral peduncles, in the pyramidal bundles of the bulbus and medulla and in the n. tibialis.

8. Chronaxie in man. In man, the most numerous and most interesting investigations we owe to Bourguignon (1), who made use of the huge clinical material he had at his disposal in the Hospice de la Salpêtrière in Paris. The experiments were performed both on normal and pathological subjects.

In physiological conditions, Bourguignon discovered a peculiar relationship between the chronaxie of a given group of muscles and their function. All the muscles contributing jointly to the same function, all the synergetic muscles constitute a homogeneous group characterized by the same chronaxie. The following table gives a short abstract of Bourguignon's figures:

Chronaxie of muscles in man (Bourguignon, 1, 22, 33)

		σ
Arm	Flexors and internal head of triceps brachii	0.08–0.16
	Other extensors	0.16–0.32
Fore arm	Flexors and pronators	0.20–0.36
	Extensors and supinators	0.44–0.72
Thigh	Forward directed movements of thigh and leg	0.10–0.16
	Backward directed movements	0.44–0.72
Leg	Forward directed movements and soleus	0.24–0.36
	Backward directed movements of leg and foot	0.44–0.72
Face	Depressor muscles	0.24–0.36
	Elevator muscles	0.48–0.72

[16b] B. A. Houssay and J. Guglielmetti (104) found in 8 experiments out of 13 the same neuro-muscular heterochronism during curare like intoxication of *Leptodactylus* by means of snake venom. The Lapicque theory of curarization has been criticized by Vogel (197). Cf. also K. Umrath (191) and C. F. Watts (198).

As we can see, Bourguignon's law puts in one and the same category muscles which are considered as antagonists by the anatomists: internal head of the triceps brachii and flexors of the fore arm, soleus and muscles that produce a forward directed movement in the leg and foot. According to Bourguignon, these muscles have the same chronaxie as their antagonists because they act at the same time. They work jointly in the same movement. They moderate the movement of their antagonists. It can be noticed that in the upper limb, the flexors have all a shorter chronaxie than the extensors. But we know that all flexion movements are quicker than the extension movements. In the lower limb, the muscles which carry the limb forward are quicker and have a shorter chronaxie than those which carry the limb backwards.

These conclusions are all in conformity with the general rule: quick movement, short chronaxie. Of the muscles of our face, those whose contraction determines an expression of joy have a longer chronaxie than the depressor muscles which express the sorrow. Moralists might conclude that pain comes more quickly than pleasure.

The differences between chronaxie of flexors and extensors have also been found by Bremer and Cambier in the turtle's muscles (35).

In the newborn child, Bourguignon and Banu (16, 17) found a chronaxie about ten times longer than in the grown-up. The motions of babies are in fact much slower than those of adults. In the new born child an heterochronism exists between motor nerves and muscles. Both get the same chronaxie at the moment the child begins to walk (15th month after birth). (Bourguignon, 25.)

The chronaxie of sensitive nerves has been measured in man. As a rule, the chronaxie of a sensitive territory is the same as the chronaxie of the underlying muscles. It looks as if the body were divided in metameres in each of which the quickness of excitability is constant (1, 32).

In long reflex arcs, the nervous or muscular elements have been found isochronic all along the path.

The results of Bourguignon's clinical investigations are also very interesting, but for lack of space, we can only give a short summary of them (1, 23, 26, 165).

When a muscle degenerates, the chronaxie lengthens. The same phenomenon is observed when a nerve is sectioned. This lengthening of the chronaxie explains the so-called Erb's reaction of degeneracy, looked for by the clinicians in their electrodiagnosis. One knows that in this reaction, faradic excitability vanishes before galvanic

excitability. Constant current is still active, when induction shocks are no more efficient. Formerly, these two forms of excitability used to be dissociated. At present, one would say that the muscular chronaxie is lengthened and that the quickness of excitability is too small to answer such a short stimulus as the induction shock. The constant current remains efficient because its passage has a long duration. The degenerated muscle shows indeed something similar to what has been observed in sluggish muscles, plain muscles for instance.

The muscular chronaxie lengthens progressively in Wallerian degeneration, which follows the section of the nerve. And this is in relationship with the muscular alteration: the amount of sarcoplasm increases and the contraction becomes slower.[17]

As to the diseases of the central nervous system, they may or may not influence the muscular chronaxie. Generally speaking, a considerable increase of the chronaxie is observed in all muscles immobilized by contracture or paralysis. Their chronaxie can be as long as seventy times the normal.

On the contrary, the chronaxie remains normal when the muscle remains capable of frequent contractions, and when consequently its nutrition remains good enough. Such is the case in shivering and in athetosis.

A remarkable fact is that in a progressive nervous disease, all the muscles of the same chronaxie are attacked at the same time by the pathological process. To similar physiological aptitudes correspond similar morbid aptitudes. The same remark can be made for the effects of some poisons. It is long known that lead, arsenic, alcohol strike in preference certain groups of muscles. These groups are composed of muscles that have all the same chronaxie, that is, muscles that act conjointly. Lead for instance assails the territory of the N. radialis, whose chronaxie is half a thousandth of a second.

9. Chronaxie of nervous centres.[18] Several researches have been performed on the chronaxie of the brain. A. Rizzolo made a series of experiments on the chronaxie of cerebral motor centres in the dog. He found that in every region of the motor cortex, one point shows a

[17] Lengthening of the muscular chronaxie of the frog occurs several weeks only after nervous section (Apostolaki and Dériaud, 15; Bourguignon, 24). According to Bremer and Gerard (37, 38) muscles of the frog do not degenerate when the motor nerve has been cut. See also Watts (198) and J. E. Abelous and H. Lasalle (11).

[18] See also below: *Iterative nerves.*

shorter chronaxie than every other point. That point he calls *optimal motor point.* The chronaxie of the grey cortex has the same value as the chronaxie of the subjacent white substance. The action of some agents, pharmacological and physical, has been studied by the same author (177 to 188).

H. Cardot, J. Régnier, D. Santenoise and P. Varé (49, 50, 51, 52, 53 and 54) investigated the influence of the vagotonic state, vagotomy, thyroidism, muscular activity, atropin, eserin, etc., on the chronaxie of the *gyrus sigmoidus* of the dog.

By means of the chronaximetric method, A. and B. Chauchard were able to establish the reality of motor localizations in the mesocephalic nuclei of fishes and frogs (58, 69).

The nervous centres have a tonic, reducing effect on the chronaxie of motor nerves in the frog. That tonic effect is of reflex origin. A frog, deprived of its skin shows the same longer nervous chronaxie as does the frog whose sciatic nerve has been cut from the centres (L. and M. Lapicque, 118, 136).

10. Chronaxie of sense organs. Here are some of the figures actually collected:

a. Taste (Man): Apex of the tongue: 1.4 to 1.8 σ. Region of lingual V: 0.3 to 0.5σ.

The existence of two different chronaxies is in agreement with the different innervation of both regions (nerves VII and IX). (A. and B. Chauchard, 68.)

b. Vestibular nerve (Man): 14 to 22 σ. (Bourguignon and R. Déjean, 30.) The vestibular nerve is associated with the sympathetic system whose chronaxies are generally long (see below).

c. Retina. G. Bourguignon and R. Déjean (27, 28, 29, 174) ascribed two different chronaxies to the human retina: the shortest one (1.2 to 1.8 σ) corresponds to the stimulation of the rods which are quick elements; the longest one (2.1 to 3 σ) to the cones, which react more slowly. Pathological conditions may affect the chronaxie of the retina and of the optic nerve.

11. Chronaxie of sympathetic and parasympathetic nerves.[19] N. Kleitman and Chauchard (106) measured the chronaxie of iridodilator and iridoconstrictor nerves in dog, cat and rabbit. They found the following figures:

[19] See also below, *Iterative nerves.*

Dilator; preganglionic	2.3 to 2.6 σ
Dilator; postganglionic	1.3 to 1.6 σ
Constrictor; preganglionic	0.8 σ
Constrictor; postganglionic	0.25 σ

Thus the chronaxie of postganglionic fibres is shorter than the chronaxie of preganglionic fibres.[20]

12. Poisons or drugs and chronaxie. Many papers have been published on the application of chronaxie to the problems of pharmacology.[21] All of them cannot be abstracted here. We have referred above to cardiac actions and to curarization. A few other researches must be referred to on account of their general interest: curare is a poison for striated muscles; it attacks only slightly the heart and plain muscles, whose chronaxie is longer: curare is a poison for quick organs (L. Lapicque, M. Lapicque and C. Veil, 124, 128, 132, 150). Other poisons attack slow organs and modify their chronaxie; they are generally more active on the heart, smooth muscles and snail's foot than on voluntary striated muscles. Such are atropin (often called curare of smooth muscles, Lapicque), quinine and its derivatives (M. Lapicque and H. Fredericq, 99), digitalin (Z. Gruzewska and M. Lapicque, 101, 119), strophantin (L. and M. Lapicque, 135), spartein (Mercier and Cherbuliez, 166), biliary salts (L. Lyon-Caen, 163), etc.

The poisons can thus be classified according to the quickness of action of the organs they first attack (Lapicque).

13. Chronaxie and imbibition of tissues. Lapicque and his co-workers have discovered a suggestive relationship between the quickness of a tissue excitability and the permeability of membranes in that tissue (113, 116, 117, 120, 132, 148).

According to Nernst's theory of excitability, excitation is considered

[20] On the contrary, V. Tedeschi (190) ascribes the same chronaxie to the pre- and post-ganglionic fibres innervating the bladder, bloodvessels and the retractor penis.

[21] Action of cocain and syncain on nerve (L. and M. Lapicque, R. Dériaud and Laugier, 73; Cardot and Régnier, 45, 46, 48). Action of caffein on the medulla (M. Lapicque, 115). Action of adrenalin, atropin, baryumchlorid on striated and smooth muscles (Lapicque, M. Florkin, 85, 87; Obré, 171). Action of atropin on vagus (A. and B. Chauchard, 63). Action of chloroform, morphin and chloralose on nerve (Chauchard, 55 and 56) and on centres (70). Action of strychnin on central nervous system and nerve (Lapicque, Bremer and Rylant, 40, 41, 42 and 43). Action of scopolamin and eserin on muscle (Marinesco and Bourguignon, 164). Changes in saline composition of heart perfusing fluids (Hansen and Schroeder, 103; Verryp and Colle, 195, 196; W. Dulière, 80) etc., etc.

as caused by changes in the distribution of ions on each side of a semi-permeable membrane, or at least along the limits of different colloidal phases.

If the excitability is quick, or if the chronaxie is short, we must admit *a priori* that the division of ions is also quick. That implies an easy permeability of colloidal media, which must not be a hindrance to the rapid migration of ions.

A longer chronaxie on the contrary could be explained by a less great permeability.

But how is it possible to appreciate the degree of permeability of the tissues? By the amount of imbibition. And the imbibition is measured by the weight of the tissue dipped into a given solution. If the Nernst hypothesis be right and if this way of reasoning be correct, one must expect to find that agents which increase the tissue imbibition diminish the chronaxie. And conversely the quickness of excitability should diminish if the grade of imbibition be low. This is in fact what happens, as found by Lapicque and others:

MUSCLE	ACTION OF	IMBIBITION	CHRONAXIE
Striated	Eserin Veratrin Nicotin K (known as increasing the permeability of membranes) Juice of *Amanita muscaria* Rise of temperature	Increased	Shortened
Striated	Curare Spartein Ca (known as decreasing the permeability of membranes)	Decreased	Lengthened
Striated	Atropin	Unchanged	Unchanged
Slow muscles (smooth, heart)	Atropin	Decreased	Lengthened

(Papers by L. and M. Lapicque, M. Nattan Larrier, 117, 120, 132, 149, 169.)

14. Chronaxie and pH. From this viewpoint, investigations have been started on the influence of pH on the chronaxie of several tissues. An increase of the H ion concentration is generally supposed to favor the muscle imbibition. Lowering the pH must shorten the chronaxie. In fact, some discrepancies are manifest in the results found by different experimenters: for a pH ranging between 5.4 and 9.2 Henri Fredericq found that lowering the pH causes a lengthening of the chronaxie of the perfused turtle's heart (93, 94). On the other hand, Bourguignon, Turpin and Guillaumin (34), Bourguignon and Haldane (31) observed that, in man, the chronaxie of voluntary muscles is longer in the state of alcalosis caused by voluntary hyperpnoea.

H. De Waele (77) and J. Beerens (18) found also in the frog that after an injection of an acid in the dorsal lymphsac, the chronaxies of both striated muscle and heart are shortened. Sodium bicarbonate has the reverse action.

Mmes. Lapicque and Nattan-Larrier (148) have produced variations of pH in the fluid in which striated muscles of the frog were dipped. The range of pH was very large: from pH = 2 to pH = 10. They weighed the muscles in different pH conditions, and they observed that the imbibition passes through a minimum, located at pH = 4. Above and below this number, the curve rises. As to the values of chronaxie, they reach a maximum located at the same point, pH = 4. Thus, the theory is found accurate.

The imbibition curve of a muscle has the same shape as the imbibition curve of gelatin accepted by J. Loeb.

A remarkable fact to which we must point, is that pH = 4 corresponds to the grade of acidity which Ganström considers as the isoelectric point of myosin (contested).

Now, at their isoelectric point proteins have a tendency to flocculate and it could readily be admitted that this reaction of the colloidal equilibrium diminishes the permeability or the imbibition power of the colloids. Thus, a physico-chemical explanation of the observed phenomena is foreseen.

15. Iterative nerves. So far, we have only considered organs which answer by an excitation to a single stimulus, induction shock, make of constant current, or condenser discharge. We have seen on the other hand that isochronism is a necessary condition for the transmission of excitation from nerve to muscle. The whole theory of curarization is based on that hypothesis.

Now there are nerves, belonging to the vegetative systems, which

can only be excited when the stimulus is applied several times. A single stimulus is in such cases inefficient. Such nerves are for instance the vagus and the vasomotor nerves. Lapicque has called them *iterative* nerves because they do not react unless excited several times. Faradization alone is efficient.

The excitability of these nerves cannot be characterized by two parameters only, the threshold intensity and the length of the stimulating current, like that of the tissues we have studied so far.

For the measurement of that iterative excitability, the following values must be known:

1. The threshold intensity or rheobasis.
2. The threshold duration (corresponding to twice the rheobasis) or chronaxie.
3. The frequency of the stimulus, that is the number of stimuli in a second.
4. The absolute number of stimuli.

If two of these values remain invariable, the two others vary in function of one another.

Thus, if the frequency and number of stimuli are stable, we can, by means of intensity and duration, measure the chronaxie, as shown in this table:

ANIMAL	ITERATIVE NERVE	CHRONAXIE OF NERVE	CHRONAXIE OF TERMINAL ORGAN	AUTHORS
		σ		
Frog	Sciatic (sensory fibres)	0.3		L. and M. Lapicque (130b)
Toad	Sciatic (sensory fibres)	0.8		
Dog	Chorda tympani	0.4		A. and B. Chauchard (57, 58b.)
Frog, dog	Vasoconstrictor	2	av. 100σ	L. and M. Lapicque (137), A. and B. Chauchard (59, 60)
Dog	Vasodilator	2		A. and B. Chauchard (61)
Frog	Vagus (heart)	2		L. Lapicque and I. Meyerson (145)
Dog	Splenic	3.2	1500	A. and B. Chauchard (66, 67)
Frog	Vagus (stomach)	10	500	L. and M. Lapicque (112, 126)

But in certain cases, the chronaxie of the terminal organ to which the iterative nerve leads can also be measured. This is the case for the walls of the blood vessels, for the spleen and for the frog's stomach. One can see that the chronaxie of the nerve and that of the terminal organ are not in agreement. There exists a thorough heterochronism between them. The chronaxie of the nerve is much shorter than the chronaxie of the organ.

This difference can be explained in the following way. To allow the transmission to take place in spite of the heterochronism, there must be a summation in the terminal organ, a phenomenon similar to the phenomenon of *latent addition*, well known to every physiologist.[22]

Therefore, the stimulus must be repeated, and the nerve is an iterative one.

The chronaxie of sensitive fibers is short. In fact, sensitive nerves are iterative. Thus, it becomes highly probable that the nervous reflex centres to which these nerves lead, have on the contrary a long chronaxie (Lapicque, 130).

Many more things could be said about the measurement of excitability by chronaximetric methods, if space permitted.

I have simply tried, with as little mention as possible to mathematics, to sum up the researches that express the effort to change our notion of excitability and our methods of measuring it. I hope I have succeeded in demonstrating the theoretical and practical value of chronaxie.

Thanks to chronaxic methods, we now begin to understand that a time value, the chronaxie, which indicates the speed of a tissue excitability, enables us to forsee all the other rates of action of the organ under consideration: quickness of muscular contraction, speed of nervous conduction, duration of nervous negative variation, not to speak of other theoretical conclusions to which this study might lead, conclusions I have only briefly sketched.

I believe that in future, we shall have to consider, whenever we are concerned with excitability, the duration and not only the intensity, the relative quickness and not the threshold. It indeed a revolutionary change will be in the traditional way of thinking of some physiologists, who perhaps will find it difficult to get rid of ancient formulae. But this change will, I feel sure, be advantageous and fruitful.

[22] For lack of space, the laws of summation related to chronaxie cannot be discussed here in every detail. (See the work of Lapicque and his pupils) (64, 65, 67, 71, 111, 112, 130b, 172).

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THE INFLUENCE OF DIET ON THE STRUCTURE OF TEETH

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It is perhaps correct to say that only recently has the subject of diet and its influence upon dental structure been recognized as important. This recognition of its significance is largely due to investigations carried out during the past eleven years which indicate that there is a definite relation between defective dental structure and caries and that the structure of teeth can be controlled by diet.

Perfect and imperfect or hypoplastic teeth. The adoption by different investigators of different standards of normality has led to differences of opinion as to the extent to which defects commonly exist. For this reason it is necessary at the outset to state as clearly as possible what is meant by perfect and imperfect, defective or hypoplastic structure, and to discuss the use of the term "normal" as applied to the structure of teeth.

It will be generally agreed that there is an ideal structure of enamel and dentine, as of other tissues of the body. The enamel is a hard, brittle and semi-translucent substance which, to the naked eye, has a smooth, lustrous and whitish appearance. Histologically it is non-cellular and is built up of prisms united by densely calcified intermediate substance, and shows little or no pigmentation under the microscope. The dentine (or ivory) is a yellowish white, translucent substance which microscopically consists of a non-cellular homogeneous material traversed regularly by branched dentinal tubes which contain organic matter, including the processes of the odontoblasts, nerve fibres, and some tissue fluid. (See fig. 1.)

The outside of a newly erupted tooth is covered by a thin membrane known as Nasmyth's membrane and the inside is filled with pulp—a soft tissue composed largely of cells, blood vessels and nerves.

Only very obvious abnormalities of structure have as a rule been described in dental literature as defects. These gross defects, which include pits and grooves in the enamel or the entire absence of enamel over certain areas, have been designated as hypoplasia and are said to occur in about 2.5 per cent of deciduous (1) and about 10 per cent of

permanent teeth. Sections of such teeth show deeply pigmented lines in the enamel, known as brown striae of Retzius, and corresponding rows of interglobular spaces in the dentine, often called Owen's lines. These "spaces" are obviously defects and are actually areas of uncalcified matrix, as will be seen below. In young growing teeth there is always a portion of uncalcified matrix—the odontogenetic zone—bordering on the pulp and penetrated by the processes of the odontoblasts. In this layer the deposition of lime salts occurs in the first place in the form of rounded masses or calcospherites. The odontogenetic zone in a well calcified tooth is thin, for the calcospherites coalesce soon after being deposited, forming a more or less homogeneous structure; but when calcification is imperfect the odontogenetic zone is wide and irregular and the calcospherites do not coalesce leaving areas of uncalcified matrix which are known as "interglobular spaces." In general it may be said that defects corresponding in time of development to the defects of the dentine are found in the enamel calcified at the same time.

In the examination of a large number of ground sections of human teeth, the first results of which were published in 1923 (2) and in more detail in 1927 (3), M. Mellanby found that the gross type of defect referred to above as being found in about 2.5 per cent of human deciduous teeth formed only a small part of the whole, and that in reality over 80 per cent of such teeth were imperfect or defective in structure. The defects varied from slight roughnesses and discoloration of the enamel surface, with slight pigmentation of the enamel and a few scattered interglobular spaces when seen in section, to very rough enamel surface and, when seen in section, diffuse pigmentation of the enamel with numerous enamel fibres and many large interglobular spaces throughout the greater part of the dentine. (See fig. 2.)

The word "hypoplasia" has been extended by M. Mellanby (2) (3) to include all these defects in macroscopic and microscopic appearance and should not be limited simply to those defects so readily seen with the naked eye. Such defects as obvious pits and grooves, previously recognised as occurring in not more than 10 per cent of permanent teeth and 2.5 per cent of deciduous teeth are referred to here as "gross" hypoplasia. Teeth showing "gross" hypoplasia often have less defective microscopic dentinal structure than teeth with no such obvious external defects. (Compare figs. 3 and 2.)

All grades of imperfect structure, one grade merging into the next, were found by M. Mellanby in her investigations, but to simplify

classification she divided the defects into three groups, slight hypoplasia, hypoplasia and severe hypoplasia (including "gross" hypoplasia). The teeth not classified under one of these headings were considered as perfect or normal in structure, the term normal thus indicating a well calcified tooth in which the enamel is smooth and little pigmented and the dentine free from interglobular spaces when examined under the microscope. It will be seen that the term normal is *not* used to denote that type of structure most commonly found in human teeth. Since interglobular spaces in human teeth have been shown to be so general, some dental authorities have suggested that they are not an indication of defect. On the other hand, if it is agreed that gross departures from the ideal structure of enamel and dentine constitute defects and that slight departures differ in degree only, and not in kind, it must surely be admitted that any such departure from the standard of perfection constitutes in essence a defect, though it may be considered as of so slight a nature as to have little or no practical significance. For the moment the practical significance is of no concern, but the term "defect" or "hypoplasia" has been used to indicate any departure from the ideal structure. This point of view is supported by the fact that although the ideal structure of teeth is relatively uncommon in human beings in England, it can be readily produced in experimental animals as also can the varying degrees of structural defect seen in human teeth classed as defective. Another argument has been raised in favour of regarding interglobular spaces as a normal development in teeth, namely, the commonness of these in the teeth of cetacea and horses. Cetacea are mammalia modified by reversion to the marine habit of life whose teeth when present are often very much altered. These animals, however, occupy such a position in nature that arguments based on the structure of their teeth can be of little significance. Moreover, the spaces in their dentine, according to the late J. Howard Mummery (4), resemble the granular layer of Tomes rather than interglobular spaces. In comparing interglobular spaces with the granular layer of Tomes he says, "The fact that the tubes of the dentine communicate with the spaces of the granular layer, while in the larger spaces they form no such communication, would apparently point to the explanation that the spaces of the granular layer represent a normal and functional structure, while the larger spaces are due to a defect in calcification." In the case of horses, these are often fed on diets calcu-

lated, according to experiments on dogs, rabbits and rats, to be described later, to produce defective structure of their teeth.

Fig. 1

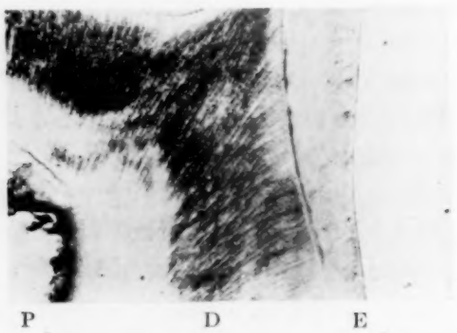

Fig. 2

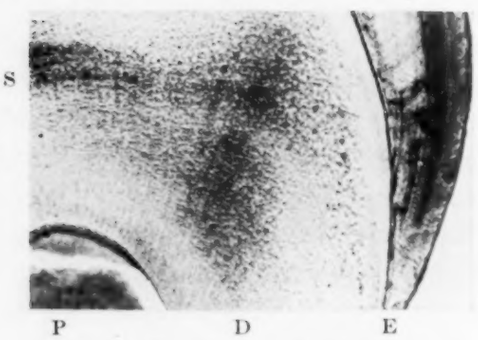

Fig. 3

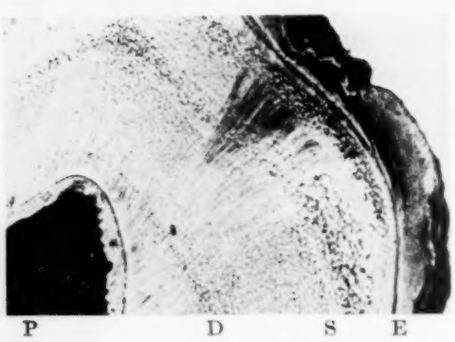

Plate I. Photomicrographs (× 30) of 3 children's deciduous molars.

Fig. 1. 1st molar. Nearly normal (i.e. perfect) in structure. Very slight pigmentation of enamel. No interglobular "spaces" in dentine.

Fig. 2. 2nd molar showing severe hypoplasia. Deeply pigmented enamel. Large numbers of interglobular "spaces."

Fig. 3. 2nd molar. Showing "gross" hypoplasia. Deficient and very irregular enamel. Many interglobular spaces in dentine some grouped as "Owen's lines." Calcification of dentine not as defective as in figure 2.

The structure of human deciduous teeth. The terms hypoplasia and normal having been defined, figures will be given to show the extent of defect found in the large survey made by M. Mellanby (3). The teeth were all examined microscopically after being ground down and their minute structure was correlated with the external appearance of the enamel which was carefully examined for any roughness and discolouration. As has already been mentioned, all grades of defect from slight hypoplasia to severe hypoplasia were found. For the purpose of analys-

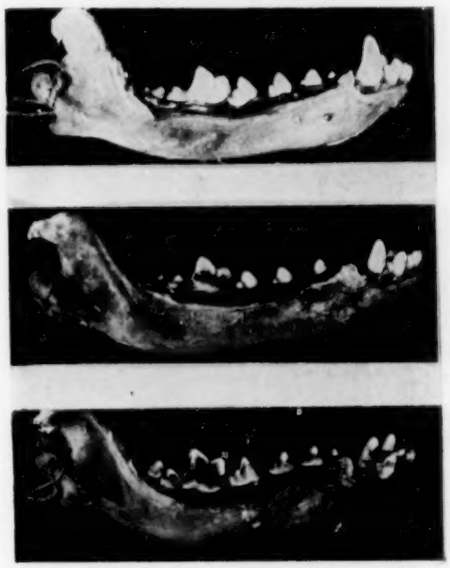

Fig. 4

Fig. 5

Fig. 6

Plate II. Photographs of 3 lower jaws of puppies, members of the same family and dieted over the same period; the same basal diet which included oatmeal.

Fig. 4. Addition to diet—*Cod liver oil*—teeth perfect (normal).

Fig. 5. Addition to diet—*Olive oil and calcium carbonate.* The extra calcium carbonate has somewhat improved the teeth as compared with the control figure 6.

Fig. 6. Addition to diet—*olive oil*, teeth very badly calcified (hypoplastic).

ing the material, all defects were grouped under the three headings, slight hypoplasia, hypoplasia and severe hypoplasia according to their microscopic appearance. In the tables below hypoplasia and severe hypoplasia are classified together (table 1). It will be seen that only 14 per cent of the teeth can be regarded as normal in structure.

Table 2 shows the percentage of microscopical defects in the deciduous teeth of children calculated from the foregoing results.

The next table shows a comparison between the amount of defect in

Fig. 7

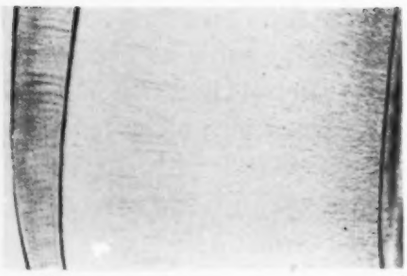

Fig. 8

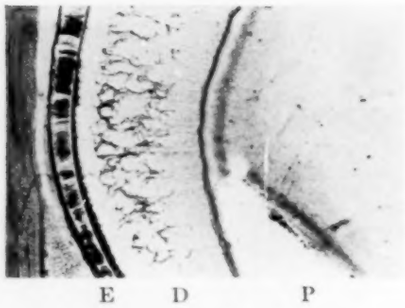

Fig. 9

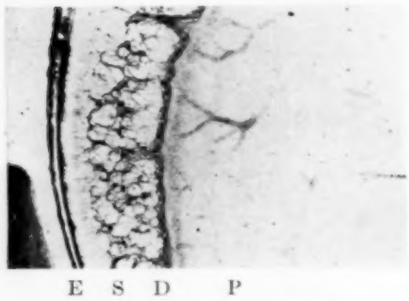

Plate III. Photomicrographs (× 40) of sections of crowns of lower carnassials (cervical region) of 3 puppies, members of the same family and dieted for the same period. The same basal diet which included oatmeal as the cereal.

Fig. 7. Addition to diet—cod liver oil and *extra* oatmeal. The enamel and dentine are thick and there are no interglobular spaces in the dentine. The tooth is normal (perfect) in structure. The oatmeal effect is completely antagonized.

Fig. 8. Addition to diet—olive oil. Enamel and dentine thin. Enamel pigmented and dentine with interglobular "spaces" (hypoplastic).

Fig. 9. Addition to diet—olive oil and *extra* oatmeal. Enamel and dentine *very* thin and poorly calcified (hypoplastic). The extra oatmeal has made the tooth worse than that seen in figure 8.

Note: In all figures E = Enamel, D = Dentine, P = Pulp, S = Interglobular "space."

the teeth obtained from dental clinics and the amount found in children's teeth obtained from private sources. The children in the private group belonged to families with more money than the dental clinic group.

TABLE 1

	NUMBER EXAMINED	NORMAL		SLIGHT HYPOPLASIA		HYPOPLASIA AND SEVERE HYPOPLASIA	
		Number	Per cent	Number	Per cent	Number	Per cent
Incisors	236	115	49	87	37	34	14
Canines	156	12	8	59	38	85	54
1st Molars	249	17	7	49	20	183	73
2nd Molars	395	5	1	27	7	363	92
Total	1,036	149	14.4	222	21.4	665	64.2

TABLE 2

	NORMAL	SLIGHT HYPOPLASIA	HYPOPLASIA AND SEVERE HYPOPLASIA
Percentage defect of all the 1036 teeth examined	14.4	21.4	64.2
Percentage defect estimated on basis of 20 deciduous teeth in mouth, consisting of 8 incisors, 4 canines, 4 first molars and 4 second molars	22.8	27.8	49.4

TABLE 3

		NORMAL	SLIGHT HYPOPLASIA	HYPOPLASIA AND SEVERE HYPOPLASIA
Percentage defect of all the 1036 teeth examined	Private	28.3	39.3	32.4
	Clinic	9.7	15.4	74.9
Percentage defect estimated on basis of 20 deciduous teeth in mouth consisting of 8 incisors, 4 canines, 4 first molars, and 4 second molars	Private	26.2	37.8	36.0
	Clinic	20.4	22.2	57.4

It will be seen that the teeth obtained from private sources were better formed than those from dental clinics. In both cases, however, it is evident that the deciduous teeth of children in England are very

defective in structure and that views previously held as to the average good structure of these teeth are erroneous.

It may be added that a similar examination of human permanent teeth, although not so comprehensive as in the case of the deciduous teeth, indicated that these were also very defectively calcified (3). Early in the present century Pickerill (5) made a careful surface examination of over 1000 teeth of savage and civilized races, from which he concluded that the enamel of human teeth is not normally quite smooth. It is traversed on its vertical aspects by minute ridges separated from each other by corresponding "furrows" which run parallel to each other in a direction at right angles to the long axis of the tooth. These horizontal lines are in some cases large enough to be visible to the naked eye. Sections of enamel from teeth with the lines well marked show the ridges and furrows in outline. These lines are called by Pickerill imbrication lines and have a close relation to the brown striae of Retzius. Pickerill considers them to represent checks in the secretory function of the ameloblasts. In the teeth of uncivilized races Pickerill found that imbrications lines, though present, were often difficult to make out, whereas in the teeth of civilized races they were very distinct and could easily be seen with the naked eye. It is interesting to note that the imbrication lines were much rarer in the teeth of Maories living under their natural conditions, and it seems probable that they may be regarded as "defects" in keeping with the foregoing results.

Experimental production of perfect and imperfect or hypoplastic teeth in animals. The most comprehensive experimental investigations so far carried out on the effect of diet on the formation of teeth are those of M. Mellanby, (6), (7), (8), (9), (10), (11) which had their origin in the work of E. Mellanby, who in January, 1918 (12), and in succeeding years (13), (14), (15), (16) published various papers showing that the problem of bone calcification centred round the presence in the diet of what he called the antirachitic vitamin (now called vitamin D), a substance having many similarities in properties and distribution to vitamin A. The early experiments indicated that there was an intimate relationship between the structure of the developing teeth and the calcification of the bones of puppies and that both were dependent for normal development on the presence in the circulation of an antirachitic vitamin, a substance whose existence was for so long questioned. It may be well, therefore, to state briefly some of the main points relating to the many investigations which have resulted finally in the actual production of the vitamin. When in 1915 E. Mellanby started working

on the subject of rickets, many views were held as to its etiology, some based on scanty experimental or clinical evidence, some based on little or no evidence, and some being merely suggestions. The results of his investigation (first mentioned in the Medical Research Council report for 1916–1917), stimulated much other experimental work on the subject, especially in England and America. In 1921 McCollum and his collaborators (17) published their first paper on the production of experimental rickets in rats on deficient diets. The existence of the antirachitic vitamin was nevertheless for a long time denied by many of the investigators, especially by Findlay and Paton and their coworkers (18), (19), (19a), (23), in the British Isles, who believed and taught that rickets was primarily due to lack of exercise and fresh air, and advocated massage in the treatment of this disease. Writing in 1922, Pappenheimer, McCann and Zucker (20) in America say, "Subsequent work in general has been unfavourable to Mellanby's conclusion. Harden and Zilva (21) in monkeys, Hess and Unger (22) in infants, Paton and Watson (23) in dogs, Hess, McCann and Pappenheimer in rats (24), Mackay and Tozer in kittens (25), Tozer (26) in guinea pigs, Zilva, Golding, Drummond and Coward in pigs (27), have all failed to produce typical rachitic lesions on diets deficient in fat-soluble vitamin." This criticism seems strange today in view of the subsequent developments and general acceptance of the antirachitic vitamin D as one of the most important of dietetic entities, and one which is now being produced on a commercial scale.

In spite of the apparent similarity in distribution and properties of the growth promoting vitamin A and the antirachitic vitamin, there was always some difficulty in accepting them as identical. For instance, E. Mellanby (13), (14), (15), (16) found that dogs might grow well and yet have badly calcified bones. Again, the growth-promoting and calcifying properties of certain vegetable fats differed greatly. Cocoanut oil, for example, tended to promote calcification but appeared to contain little or no vitamin A. Butter, which stimulated calcification very potently under some conditions, did not under other conditions influence calcification so much as it influenced the growth of animals. Nevertheless, for some years E. Mellanby hesitated to differentiate between the antirachitic vitamin and vitamin A. The differences between the two vitamins were further emphasized by the experiments of McCollum (28) on the relative susceptibility of the two vitamins to heat, and also by the work of McClendon and Schuck (29), Zucker and Barnett (30), Goldblatt and Zilva (31), Chick and Roscoe (32), and

Boas (33) which showed that green vegetables, although they had but little calcifying influence, were rich in the growth-promoting properties of vitamin A. In 1927 the artificial production of the antirachitic vitamin by the action of ultra-violet light on ergosterol (34), (35), (36), resulting directly from the original observations of Huldschinsky (made in 1919) (37), (38), that ultraviolet irradiation of the skin cures rickets, has placed this vitamin in a category of its own and it is row universally accepted as vitamin D.

In 1922 E. Mellanby (39), (40) published work which showed that although the central factor in calcification was vitamin D, yet certain foods tended to prevent calcification. Among such foods were cereals, and especially oatmeal. He provisionally suggested the word "toxamin" to indicate the anticalcifying substance present in certain foods or foodstuffs.

Since 1917 the effect of diet on the development of the teeth has been studied by M. Mellanby concurrently with the investigations of E. Mellanby on bone calcification. The results have been published from time to time in a series of papers appearing from 1918 onwards. Most of the experimental work on diet in relation to tooth formation other than that of M. Mellanby has centered round vitamin C (41). The defects produced by a deficiency of this vitamin, however, are not those commonly found in man. Other papers have also been published on the effect of other dietetic deficiencies on the teeth, but most of these investigations deal primarily with the effect of diet on the experimental production of caries (41a) and other diseases rather than on defects of structure. For these reasons the basis of this review must depend largely on the work of M. Mellanby which has involved observations on 1400 dogs and the preparation of over 2000 ground sections of their teeth and jaws, as well as many hundreds of experiments on rabbits and rats.

Experimental methods and diets. The domestic dog was largely used in the experiments described by the writer because it has a metabolism somewhat similar to that of man. Like man, the domestic dog is omnivorous and it has the great advantage of being able to live as a member of a family under the same hygienic conditions as man. It can also readily be kept in excellent health under laboratory conditions as long as desired, at any rate up to eight years. Moreover, the defects found in the dog's teeth under experimental conditions are on the whole similar to those found so commonly in man. The normal enamel of the dog's tooth, like that of man, is hard, brittle and semi-translucent. All

grades of defect, including pigmentation of the enamel and interglobular spaces in varying quantities in the dentine can be obtained at will. In the dog, as in man, under ideal conditions there is in the developing tooth a thin, regular odontogenetic zone, which under abnormal conditions becomes thick and irregular.

Any possibility that the factors controlling the structure of dog's teeth stand in a category by themselves has been excluded by an extension of the experiments to rabbits and rats and, on a small scale, to human beings, the results obtained being in all cases closely comparable to those previously found with dogs.

There is no need here to say much about the experimental methods except that the puppies were all kept under similar hygienic conditions unless otherwise mentioned. In comparing two or more diets puppies of the same litter were used, and, generally, only one factor was varied at a time. The puppies used in each experimental series were put on diet at the same time and killed at the same time. A typical basal diet found after many trials to be suitable, is as follows:

Cereal, 80 to 200 grams
Lean meat, 10 to 30 grams
Separated milk, 150 to 300 cc.
Fat, 10 grams
Orange juice (for antiscorbutic vitamin c)
Yeast (for water soluble vitamins)
Sodium chloride, 1 to 4 grams

The amount of food given to the individuals of a family was usually regulated by the amount eaten daily by the puppy with the smallest appetite. If one puppy left some food, the amount of food given to the whole family was generally cut down. It will be noticed that this basal diet consists of ordinary foods and not of the purified food elements commonly used in rat feeding experiments. The main reason for this is the impracticability of feeding large numbers of puppies over long periods of time on pure carbohydrate, fats, proteins, etc. It has a further advantage in that it avoids the assumption that all the essential elements in the diet are already known, an assumption often made in work carried out with synthetic diets of purified foodstuffs.

According to dietetic standards of say 15 years ago, the basal diet is satisfactory in respect of protein, fat, carbohydrate, and salts. Moreover, it is eaten with avidity and allows good growth and, except under special circumstances, good health at least over the required period of three to twelve months.

The presence of yeast provides vitamins B1 and B2, and orange or lemon juice provides vitamin C. The influence of vitamins A and D can be determined by varying the fat, among other things. Thus the inclusion of linseed or olive oil means the reduction of these vitamins to a low level while its substitution by cod liver oil ensures a large supply of these vitamins. The addition of cabbage when the fat is olive or linseed oil brings into the diet an abundant supply of vitamin A, while irradiated ergosterol can be used to supply vitamin D. In most of these experiments, however, the dietetic addition made to include D also included A since these vitamins are so often associated together.

It is true that a diet thus constituted has the drawback that it is difficult to exclude completely from it any given factor, but, on the other hand the amount of the particular food factor under investigation can be so greatly varied that the results obtained are as a rule definite, especially if the factor plays an active part in tooth calcification.

So far as these experiments go, and there does not seem to be any experimental evidence of a contrary nature, diet is seen to influence the formation of teeth in three ways, *a*, the calcifying vitamin (vitamin D) increases and indeed seems to control the actual calcifying process; *b*, cereals act in the opposite way and specifically interfere with tooth calcification; *c*, since teeth are made up so largely of calcium and phosphorus, the diet must contain sufficient of these elements, though the minimum requirements depend largely on *a* and *b*. One other condition, apart from diet, greatly influences tooth formation, namely, the irradiation of the animal's skin by some source of ultra-violet radiation such as sunlight or a mercury-vapour lamp The effect of the ultra-violet irradiation is the same as that of the addition of vitamin D to the food. Some consideration of its action will be given when the food factors have been described. The effect of the majority of foods on the structure of the teeth can be explained by their vitamin D, cereal or salt content and, although it is true that much remains to be discovered as to how the specific food ingredients act, it is probable that at least the main factors in food which influence tooth formation have been distinguished.

A more detailed account of each of the food factors having a specific influence on the calcification of teeth will now be given.

THE EFFECT OF THE CALCIFYING OR ANTIRACHITIC VITAMIN D. Using the basal diet mentioned above, it was found, with one exception, that when the fat of the diet consisted of a vegetable oil defective structure of the teeth resulted (figs. 6 and 9). The exception was cocoanut oil.

Many vegetable oils were tested, including linseed, olive, palm kernel, babassu, cotton seed, cocoanut, peanut (arachis) and others. Prior to 1919 peanut (arachis) oil resulted in the development of fairly good teeth, but in all later experiments it produced very bad calcification. It is possible that the method of preparation of the oil during the War period was different from that used today. When cod liver oil was given even in small quantities instead of a vegetable oil, perfect calcification resulted (figs. 4 and 7). Two cubic centimeters daily of this oil usually resulted in excellent structure even when the cereal of the diet consisted of oatmeal. Butter and suet improved calcification greatly, and it was found that butter gave better results than butter fat. Lard had some calcifying action but none of the samples tested had as much as suet. Heating fats to 120°C. in the presence of oxygen ultimately destroyed their power to promote calcification. Twelve hours of such treatment in the case of butter and 72 hours in the case of cod liver oil had this effect. It is to be noted that in all comparative experiments for testing different fats the amount and variety of proteins, carbohydrates and salts (including of course calcium and phosphorus) were identical, the only difference in the diets being the type of fat eaten, and yet the teeth were perfectly formed, or normal, in some cases (figs. 4 and 7) and very defective, or hypoplastic, in others (figs. 6 and 9). The simplest interpretation of these results seems to be that some fats are rich in a fat soluble vitamin now called vitamin D, which enables the ameloblasts and odontoblasts to make use of the circulating calcium and phosphorus and other salts and so to incorporate them in the developing enamel and dentine.

Experiments made to test some of the ordinary foods suggest that any tooth-calcifying properties present depend primarily on their vitamin D content. For instance, egg yolk, which is very rich in vitamin D, greatly stimulates tooth calcification. Egg white, on the other hand, is deficient in the vitamin and has little or no effect on calcification. Lean meat, which always contains some fat with its associated vitamins between and in the fibres, results in improved tooth structure. Green vegetables slightly improve calcification, but as previously mentioned (p. 554) though rich in vitamin A they are usually poor in vitamin D, particularly in the winter months. It must be noted that vegetables are not readily digested by dogs. In rabbits, which are accustomed to green vegetables, it was found by M. Mellanby and Killick (42) that cabbage added to a diet of bran, oats and calcium carbonate resulted in bad calcification of the teeth and bones unless the oats and bran mixture

was reduced and the cabbage greatly increased, when perfect or nearly perfect calcification might result. Whole milk in sufficient quantities in relation to the other factors of the diet, results in good calcification, as would be expected, for vitamin D is present in milk, varying in quantity with the food and environment of the cow providing the milk (43). The calcifying effect of butter though made from the milk is, however, definitely smaller than that of the milk itself. This is partly due to the fact that all the fat is not removed from the milk in the preparation of butter. Butter also acts much better as a calcifying agent when it is associated with a large quantity of calcium, such as is present in milk. For this reason also butter, which contains some calcium in association with the casein in it, is more active than butter fat prepared by dissolving butter in petrol ether and so removing the calcium. Again, if extra calcium, either in the form of calcium carbonate, calcium phosphate or calcium acid phosphate, is added to a diet containing butter, but otherwise deficient in calcium, the beneficial effect on calcification of the teeth is greatly increased. There is always a possibility that butter fat has associated with it, not only vitamin D, but also some anticalcifying factor, and that this latter factor is neutralised by the additional calcium, as is apparently the case with the toxic factor in cereals, a point which will be discussed later. In any case, a given quantity of milk is greatly superior to the corresponding quantity of butter which can be made from it, and the effect of butter is enhanced by the addition of extra calcium. Margarines prepared from most vegetable oils are devoid of calcifying properties, unless as is the case in some of the margarines prepared by a firm in England, the fat-soluble vitamins extracted from cod liver oil are added to make the calcifying powers equal to those of butter. Some of the pulses (peas, lentils and beans) have also been tested but have not been found to stimulate the formation of good teeth and are therefore probably devoid of vitamin D.

The experiments of M. Mellanby and E. Killick (42) on rabbits confirm the experiments on puppies. The basal diet used for these animals was as a rule bran, oats and calcium carbonate. The addition of cod liver oil or vitamin-containing extracts of cod liver oil, egg yolk, milk and other vitamin-D-containing substances, resulted in normal calcification, whereas substances lacking or relatively deficient in vitamin D, such as egg white and white turnip, resulted in hypoplastic teeth.

In 1922 McCollum, Simmonds, Kinney and Grieves (44) published a paper describing the condition of the teeth of rats in which rickets had been experimentally induced. These experiments will be referred

to again later since the teeth were examined primarily for the presence of caries and other dental diseases. Specimens were prepared by boiling and macerating. Sometimes the teeth were found to be brittle and easily fractured. Osteodentine was occasionally noted and hypoplastic enamel is described.

A full account of careful experiments carried out by Toverud between 1923 and 1926 is given in a paper published as a supplement to Den Norske Tandlaegeforenings Tidende 1926 (45), (46). On the dental side the work deals mainly with the calcium content of the incisors and molars of rats fed on various diets, the chief variables in which were the amount of calcium and vitamin. An analysis of the ash, calcium, phosphorus and magnesium was made independently in the incisors and molars of rats fed on the varying diets. The standard diet was composed of 92 per cent wheat, 5 per cent butter fat and 3 per cent salt mixture. (For a calcium-poor diet the calcium carbonate in the salt mixture was reduced by two-thirds. For a vitamin-poor diet 3 grams of butter fat were replaced by coco-fat. For a vitamin-rich diet he gave wheat flour 73.5 per cent, vitamin-free casein 14.5 per cent, coco-fat 8 per cent, cod liver oil 2 per cent, yeast extract 0.5 per cent, sodium chloride 1.5 per cent. For a high oatmeal and low calcium and vitamin diet he gave oatmeal 84 per cent, skim milk 10 per cent, yeast extract 5 per cent, sodium chloride 1 per cent.)

Compared with the findings in the teeth of animals fed on the standard diet, his analyses showed that with a calcium-poor diet the ash, calcium and phosphorus were reduced while the magnesium was increased. These changes were still more pronounced with a calcium and calcifying vitamin poor diet, while with a calcium poor and very rich vitamin diet practically normal values were obtained. With high oatmeal and low calcium and vitamin the ash, calcium and phosphorus were well below the standard while the magnesium was above it. Feeding defective diets to rats having already fully formed molar teeth when the diets were started, resulted in changes in the chemical composition of such teeth. Apart from the chemical analysis of the teeth he noted that with calcium and vitamin deficient diets, *a*, salivary calculus was often seen on the teeth; *b*, lesions of the attaching tissues were fairly common.

On the whole Toverud found that the incisors were more influenced by the diets than the molars, and that the changes were somewhat more pronounced in the second generation than in the first. Moreover his experiments indicated that the chemical composition of rats' molars, full grown at the start of the experiment, was changed.

In 1927 Orban (47) described the effect on rats' teeth of adding different substances to a diet of wheat. He found big differences in the rate of growth of the incisors as well as in the structure of the developing dental tissues. His main conclusions were that milk, cod liver oil and calcium greatly improve calcification. Adding vitamin C or B to the control diet had little or no effect. These results, as far as they go, confirm the results found with dogs and rabbits, mentioned above.

The evidence available in the case of the development of children's teeth shows that the effect of a fat-soluble vitamin in the diet is to produce perfect or nearly perfect calcification just as in the puppy experiments.

THE INTERFERING EFFECT OF CEREALS AND CEREAL PRODUCTS. In his Oliver Sharpey lectures in May 1922 (39) and at a meeting of the British Medical Association in Glasgow (40), E. Mellanby discussed the rachitic action of cereals, especially of oatmeal. He has published several papers (39), (40), (48), (49), (50) on the effect of cereals on the production of rickets in dogs and rats, and has suggested that they may perhaps contain some anticalcifying substance which he has provisionally called a toxamin (51). P. M. Holst, in a Norwegian paper (52) published in September 1922, stated that rats and rabbits develop rickets when fed on boiled oats and cabbage. He continued these studies and in 1924 (53) also came to the conclusion that this condition is produced by some unknown anticalcifying factor. In 1923 the effect of cereals on the development of teeth in puppies was described in a paper by M. Mellanby (10). It was found that the greater the amount of cereal eaten, other things being equal, the greater was the tendency to the production of badly calcified teeth. If, for instance, the basal diet above described (p. 555) contained 200 grams of oatmeal (fig. 9), the teeth were worse formed than if it only contained 100 grams of oatmeal (fig. 8), all other ingredients being constant and the diet remaining deficient in vitamin D. It may be suggested that this was due to the faster rate of growth of the animal eating the larger quantity of cereal and to the fact that the calcium-retaining properties of the diet were not sufficiently potent to bring about the absorption and storage of the extra calcium and phosphorus needed for the more rapidly developing tissues. This explanation is negatived by the fact that if equal quantities of different cereals are eaten as part of the diet by animals of the same litter, different degrees of tooth calcification result. In such cases the animals may grow and put on weight at the same rate, so that the demand for calcium and phosphorus on the part of the growing tissues is presumably the

same in each case. Of the cereals tested oatmeal is by far the worst for producing badly calcified teeth. Maize is also bad and is followed, in decreasing order of interfering potency, by rye, whole meal flour, rice and white flour, the last of these having usually the least effect. The germ of both maize and wheat, and possibly of other grains such as rye, contains some factor or factors interfering with calcification. The anticalcifying effect of any of the cereals can always be antagonised by the addition to the diet of sufficient vitamin D (figs. 4 and 7). One striking feature of these results, which illustrates how extremely difficult it is to base any argument as regards the power of diet to promote tooth calcification on a priori reasoning, is that those cereals which have the worst effect on the teeth contain the most calcium and phosphorus. This point is brought out in the following table which gives the amounts of calcium and phosphorus in the different cereals (Sherman, *Food Products*, New York, Macmillan, 1920).

TABLE 4

	PER CENT CALCIUM	PER CENT PHOSPHORUS	Ca:P RATIO
Oatmeal	0.069	0.392	1: 5.7
Maize	0.020	0.283	1: 1.4
White flour	0.020	0.092	1: 4.6
Whole meal flour	0.031	0.238	1: 7.7
Barley pearled	0.020	0.181	1: 9.0
Rye flour	0.018	0.289	1:16.1
Wheat germ	0.071	1.050	1:14.8
Rice	0.009	0.096	1:10.7

These figures show that oatmeal and wheat germ which experimentally produce the worst calcified teeth have far more calcium and phosphorus than the other cereals. McCollum and his co-workers (54) have stated that, as regards calcification of bones in rats, the calcium-phosphorus ratio is of great importance. This fact, however, does not explain the varying effect of cereals in regard to teeth, for although wheat germ has the highest calcium-phosphorus ratio, viz., 1:14.8, that of oatmeal (1:5.7) is by no means high and is exceeded by that of whole meal flour (1:7.7), barley (1:9.0), rice (1:10.7) and rye flour (1:16.1), none of which is nearly so effective as oatmeal in preventing good tooth calcification. Nevertheless, the addition of calcium to a diet deficient in vitamin D does materially assist calcification, especially if it is given in the form of carbonate. An adequate amount of vitamin D in the diet completely antagonises any cereal effect without additional calcium.

The anticalcifying effect of cereals cannot be explained by their carbohydrate content for oatmeal contains only 67.5 per cent carbohydrate, while white flour contains 76.4 per cent and rice 79.0 per cent. Indeed, after oatmeal has had most of its starch digested away by diastase, the residue is still capable of interfering to a considerable degree with the calcification of the teeth.

About seven out of the eight per cent of fat contained in oatmeal can be removed by boiling with petrol ether, but the residue is still as active as ever in interfering with the formation of the teeth. The remaining 1 per cent of fatty compound can be detected after saponification, acidifying and extracting with petrol ether. It is possible though not probable that this fatty residue may be the active compound, for the fatty acids prepared from saponified oatmeal are capable of interfering to some extent with the formation of the teeth. This is probably, however, not a specific effect and does not explain the whole anticalcifying properties of the oatmeal. All attempts made up to the present to isolate or even to gain some insight into the composition of the interfering substance contained in cereals have failed.

One other point in connection with the anticalcifying action of cereals is worthy of notice. Oatmeal can be boiled with water or heated in a dry state at 120°C. for 18 hours without destroying its anticalcifying properties. If, however, oatmeal be boiled with 1 per cent hydrochloric acid for 1½ hours to the achromic point with iodine, i.e., until all its starch is converted to dextrose, the neutralised product is found to have lost its power of interfering with the calcification of the teeth. The time of boiling and strength of acid are of importance in these experiments for P. M. Holst (55) has found that the rickets-producing effect of oatmeal can be demonstrated in a filtrate obtained after boiling this cereal with 0.5 per cent hydrochloric acid (time unstated). It is probable that, whereas a short period of boiling with dilute acid brings the rickets-producing substance into solution, more prolonged boiling destroys it. The improvement in the structure of the teeth produced by boiling oatmeal with acid as compared with that produced by the untreated product, is probably due to the destruction of the anticalcifying agent and is not comparable to that produced by the addition of either calcifying vitamin or calcium salts.

The question as to whether the anticalcifying action of cereals may be due to the fact that their reaction is potentially acidic has been discussed by E. Mellanby (49). The importance of acidosis in rickets has often been considered, but without experimental evidence. On the

other hand M. Jones and her collaborators (56) consider the utilisation of calcium salts to be better when the diet is potentially neutral or acid in reaction than when it contains a preponderance of base. The basal diets used consisted of bread, cooked lean beef, skim milk, pure butter fat, orange juice, salt mixture and distilled water. The salt mixture was based on the analysis of the ash of cow's milk, but phosphorus was omitted. Different proportions of calcium were used. The amount of phosphorus was varied by adding dibasic potassium phosphate and the alkalinity by adding hydrochloric acid. Although these experiments deal primarily with bone changes some mention is made by Simmonton and M. Jones (57) of the structure of teeth in relation to the acid-base ratio of the experimental diet in a paper on odontoclasia published in 1927. Owing to the fact that no details of diet are given in relation to tooth structure, it is not easy to interpret their results. They define

TABLE 5

FOODSTUFF	EXCESS OF		FOODSTUFF	EXCESS OF	
	Acid	Base		Acid	Base
Oatmeal	3.0		Milk (skimmed)		5.0
Whole meal flour	3.3		Beef	4.9	
White flour	2.7		Cabbage		18.0
Rice	2.7		Carrots		24.0
Barley	2.9		Eggs	7.5	
Milk (whole)		2.6			

odontoclasia as a lesion "which simulates human caries but differs from it both clinically and microscopically in certain respects." The coronal portion of a characteristically affected tooth exhibits stained areas, loss of tooth substance and zonation. The term odontoclasia appears to the writer to be used in some cases to indicate enamel defects similar to those which were shown by the writer to be developmental in nature. It is difficult to decide whether they consider hypoplasia and odontoclasia to be related or not. Probably the difficulty arises partly because they do not define their use of the term hypoplasia.

It is certainly true, as mentioned later in this review, that the addition of calcium carbonate to a diet containing oatmeal brings about improvement in the calcification of teeth, but so does calcium acid phosphate.

The numbers representing the acid-base ratio of the different cereals, and indeed of many other foodstuffs, appear to bear no relationship to their influence on the development of the teeth. Sherman gives the

above figures (Table 5) for the acid-base ratios of some foodstuffs whose influence on teeth formation has been investigated.

It is perfectly clear that, if these figures are correct, there can be no direct relationship between the acid-base ratio and the effect on teeth calcification of any particular food. For instance, there is very little difference between the figures for oatmeal, white flour, barley and rice, although the difference between the effect of some of these substances on developing teeth is great. Milk and eggs both greatly assist calcification and yet in the first case the ash is basic and in the second acidic in character.

THE EFFECT OF THE ABSOLUTE AND RELATIVE AMOUNTS OF CALCIUM AND PHOSPHORUS INGESTED. Whereas it is true that the normal calcification of bones and teeth can only be produced when the diet contains more than a minimum of calcium and phosphorus, it is equally certain that these necessary minima depend upon other factors mentioned in this review, the more important of which are:

1. The amount of calcifying vitamin in the diet.
2. The rate of growth of the animal.
3. The amount of exposure of the animal or certain food constituents to ultra-violet radiations.
4. The amount of anticalcifying substance in the cereals eaten.

It is obvious from what has already been said that the use of the expression "optimum calcium and phosphorus content of a diet" is to be deprecated, for the necessary amounts of these substances, particularly calcium, depend so much on other factors (10). It may be predicted with some certainty that, if there is an abundance of vitamin D in the food, or if the animal is exposed over long enough periods of time to ultra-violet radiations, the calcium-phosphorus ratios are negligible from the point of view of the development of perfect teeth, and that even the absolute amounts of calcium and phosphorus in diets made up of ordinary foodstuffs are of but little significance. Puppies given a diet containing little calcium, consisting for instance of bread, lean meat, orange juice and yeast, with no separated milk powder after the first few weeks, but with 3 cc. of cod liver oil daily develop good teeth, and very little difference is observed in their structure when extra calcium is added to the diet. If linseed oil is substituted for the cod liver oil in the above diet, the teeth are very defective, but in this case calcification is definitely improved by the addition to the diet of calcium in the form of calcium hydrogen phosphate or calcium carbonate. When, instead of bread, oatmeal forms the cereal of a diet deficient in vitamin

D (fig. 6), calcification is even more greatly improved by the addition of calcium, (fig. 5), but to ensure the production of perfect teeth a sufficiency of vitamin D (fig. 4) is essential.

Most natural food mixtures appear to contain enough calcium for the production of perfect teeth provided that sufficient vitamin D is present. Moreover, in many foods, especially the more expensive foods such as milk and egg (yolk), vitamin D and calcium are closely associated.

In dogs, therefore, the part played by the calcium and phosphorus of the food is to a great extent dependent on vitamin D and the deposition of these elements in the teeth depends largely on the available supply of this substance (10). When, however, the amount of vitamin D in the diet is very small, and especially when cereals and butter are eaten, the calcium intake plays a more prominent part and controls to some extent any influence these foods have on the structure of the teeth.

Although these facts may possibly not apply to all mammals it is probable that in general they do apply. Certainly in man the few cases investigated definitely support the suggestion. Reference has already been made to the work of McCollum and his collaborators and others (58), (59), (60) on calcification processes in rats, from which they conclude that the calcium-phosphorus ratio is of great significance. The point has been particularly emphasised by McCollum, Simmonds, Shipley and Park (58). Other investigators who have supported this point of view include Korenchevsky (59) Sherman and Pappenheimer (60). It would certainly appear that in the rat the calcium-phosphorus content of the diet affects the development of rickets. The fact, however, that even in the presence of what has been called the optimum calcium-phosphorus ratio, osteoporosis generally follows the ingestion of a diet deficient in the antirachitic vitamin is evidence that even in this animal the vitamin is the dominant factor.

M. Mellanby and Killick (42) investigating the calcification of rabbits' teeth and bones, found that diets which readily produced defective structure had a calcium-phosphorus ratio about equal to that said by McCollum and others to prevent rickets in rats.

Marshall (61) examined the heads of some of the dogs which had been used by M. Jones and her collaborators for nutrition experiments. The diets are not mentioned but he states that they varied as regards their calcium and phosphorus contents. He found variations in the shedding of the deciduous teeth and gross abnormality in the formation of some of the teeth. The dentine in one case was said to have been entirely

without calcium. Histological details of enamel and dentine defects are not given.

THE EFFECT OF VITAMIN C. Some of the earliest work carried out to determine the influence of the diet on the teeth was that of Zilva and Wells (41), who fed guinea pigs on a scorbutic diet of oats, bran and autoclaved milk. Ground and decalcified sections of the teeth were prepared and defects were seen in the pulp and dentine. In the pulp various degrees of degeneration of a type designated as "fibroid" were found, and in the dentine an irregular "osteoid" condition. Changes in the teeth were observed even in the mildest degree of scurvy but never when vitamin C was added to the basal diet.

P. Howe has also carried out much experimental work on guinea pigs and monkeys, primarily, however, with a view to establishing the relation of diet, and in particular vitamin C, to caries. He concludes that a deficiency in this vitamin leads to decalcification of the teeth.

In 1923 Toverud (45) made a histological and chemical examination of some of the teeth of Howe's guinea pigs and found the same defects that were described by Zilva and Wells.

Robb and collaborators (63) found similar changes in the teeth of guinea pigs on a scorbutic diet.

Extensive investigations by Höjer, (64), (64a) Höjer and Weston (65) and others have emphasised the importance of vitamin C in determining the structure of the teeth in guinea pigs. They summarise the defects that follow vitamin C deficiency somewhat as follows.

1. The gradual change and disappearance of the odontoblastic layer.
2. The amorphous calcification of the "predentine" and the absence of canals in this layer.
3. The previously calcified dentine becomes porous.
4. The dilatation of the vessels in the pulp.
5. The nervous and subsequent hydropic processes in the pulp tissue.

As a result of these investigations a satisfactory method was devised for the estimation of the scorbutic value of foods.

It is difficult to estimate the degree of applicability of these results to man. The lesions produced by a deficiency of vitamin C in guinea pigs are rarely found in man, partly because human beings are not as susceptible to vitamin C deficiency as are guinea pigs. So far as experiments on dogs are concerned, no evidence has been obtained by the writer to show that vitamin C plays any significant part in the calcification of teeth.

Whereas it is improbable that the actual structure of human teeth is

greatly affected by a deficient intake of vitamin C the spongy gums associated with scurvy suggest that this vitamin plays a part in some dental diseases.

THE CALCIFYING EFFECT OF ULTRA-VIOLET IRRADIATION. In 1919 Huldschinsky (37), (38) made the fundamental discovery that ultra-violet radiations of a mercury-vapour lamp striking the skin of rachitic children brought about increased calcification and cured the disease. A similar effect was produced by Hess and Unger (65a) using sunlight as a source of ultra-violet radiation. These facts were later confirmed by Chick, Dalyell, Hume, Mackay and Smith (66) in an extensive investigation undertaken on Viennese children. At first these observations on the effect of light appeared to have nothing in common with the action of the antirachitic vitamin previously described by E. Mellanby (12), (13), (14), (15), (16). Many investigations were carried out on rats by a number of workers with the object of associating the action of light and diet on calcification and growth. Among the numerous results obtained the following may be mentioned.

1. The stimulating effect on growth of ultra-violet radiations applied to rats fed on diets deficient in fat-soluble vitamins (66), (67), (68).

2. The power to promote bone calcification of foods deficient in fat-soluble vitamins exposed in thin layers to ultra-violet radiations (69), (70).

3. The development of calcifying properties in cholesterol after exposure to ultra-violet radiations (71), (72), (73).

The field was more and more narrowed down until it was finally shown in 1927 by Rosenheim, Webster (34), (35) Hess and Windaus (36) that ergosterol, a substance previously isolated from ergot by Tanret, was made into vitamin D by ultra-violet radiations. Ergosterol was shown to be present in the skin as well as in many other plant and animal tissues. Incidentally, the above mentioned investigations definitely dissociated vitamin D from vitamin A.

In 1923 M. Mellanby (74) demonstrated the improvement in the calcification of the teeth in animals exposed to sunlight or to a mercury-vapour lamp. After Steenbock and Nelson (75) had shown that foods previously without any effect on bone calcification acquired potent calcifying influences on the bones of rats after being exposed to ultra-violet radiations, the same principle was shown by M. Mellanby to apply to calcification of the teeth. For instance, olive oil which is devoid of vitamin D, acquired after irradiation the power of improving the structure of developing teeth. Thus, an inert fat becomes active. The

calcifying influence of butter is also greatly increased by irradiation. If, however, either olive oil or butter is heated to 120°C. and oxygenated and the product is then irradiated, no calcifying influence is developed as the result of the irradiation; the vitamin D contained in butter has previously been shown to be destroyed by the treatment. Thus, it would appear that heat and oxygenation not only destroy the calcifying vitamin, but also destroy the parent substance of the vitamin. E. Killick and M. Mellanby (42) demonstrated similar effects of irradiation on the teeth of rabbits.

It was interesting to discover that cereals, which have been referred to above as actively interfering with the calcification of teeth, could be made to lose their power by exposing them to ultra-violet radiations. After such treatment they actually improve calcification. If the bulk of the fat contained in oatmeal be removed by petrol ether the residue is practically unaffected by ultra-violet radiations, for it still interferes actively with the formation of the teeth, whereas if the fat extracted from oatmeal be exposed to a mercury-vapour lamp it develops a potent calcifying action on the teeth. Thus it appears that exposure of oatmeal to ultra-violet radiations does not destroy the anticalcifying substance but brings about the development of sufficient vitamin from the inactive ergosterol to antagonize completely the anticalcifying influence of the original oatmeal. This method of antagonizing the toxic effect of oatmeal is therefore different from that referred to in a previous section where it was shown that oatmeal boiled with dilute hydrochloric acid becomes relatively inert.

Although there is as yet but little known of the actual distribution of ergosterol in nature, it is probably a constituent of many foods, since after irradiation foods which were previously anticalcifying such as cereals become active calcifying agents, foods which were neutral may now stimulate calcification (vegetable oils) and foods which previously improved the teeth become even more potent in this respect (butter, milk).

THE EFFECT OF THE MATERNAL FEEDING ON THE STRUCTURE OF THE TEETH OF THE OFFSPRING. A reference must be made here to an investigation on the effect of the diet of the mother during pregnancy and lactation, on the development of the teeth of the offspring. M. Mellanby (76) found that when pregnant and nursing animals were fed on diets deficient in the calcifying vitamin, the dentine of the deciduous teeth of the offspring often contained small interglobular spaces and the enamel might be defectively calcified, indicating that the mothers had

been unable to supply from their own bodies a sufficiency of the factors necessary to ensure perfect calcification. Since the mother tends to sacrifice her own needs to those of the offspring, it is not to be expected that great defects will be found in the calcification taking place during antenatal life. More defects might be expected during lactation when the mother tends to have exhausted her stores, but most after weaning when the diet of the young is so liable to be unbalanced. Actually it is found in puppies that deciduous teeth which have developed and erupted before weaning are rarely very hypoplastic, whereas those parts of the permanent teeth which have developed and erupted after the puppies themselves have been given the same deficient diets may show severe hypoplasia.

However hypoplastic the teeth of the experimental dogs and of children may be, it is of interest to note that there is generally a band of normal dentine bordering on the enamel (Figs. 2, 8 and 9). The width of this band varies greatly in the different teeth and depends partly on the amount of the particular tooth calcified at the time at which the puppie or child becomes independent of the mother for food, that is, at the time of weaning. For instance, calcification starts earlier in the human deciduous incisors than in the molars; the incisors often have therefore a wider band of well calcified dentine than that usually seen in the molars.

THE EFFECT OF DIET ON DENTAL STRUCTURE PRODUCED IN RESPONSE TO EXTERNAL STIMULI. Up to this point we have been concerned with the effect of specific dietetic factors on the structure of the enamel and primary dentine of the developing tooth. After eruption and complete calcification of the original tooth, a reaction to some harmful stimulus, such as attrition or caries, is often found to take place. This reaction may result not only in a localised alteration in the primary dentine but also in the formation of what is known as secondary dentine. The odontoblasts, which previously were comparatively inactive, are stimulated to renewed activity and lay down more calcified material in continuity with the original dentine but usually localised to a region corresponding to the stimulated portion of the enamel and dentine.

It is easy to bring about the production of secondary dentine in dogs by rubbing the tooth with a file at intervals of two or three days. This procedure is simply a controllable method of causing attrition of the tooth. In human teeth secondary dentine is often found, sometimes resulting from attrition, sometimes in response to caries. In an examination of the secondary dentine in a large number of teeth, both of dogs

and human beings, M. Mellanby (77, 78) found that sometimes it was well calcified and sometimes it showed defects in calcification such as the presence of interglobular spaces. In some cases no secondary dentine was found in spite of evidence of attrition or caries. This reaction is obviously an attempt on the part of the living tooth to defend itself. In the experimental puppies the quantity and quality of the secondary dentine formed as the result of natural or artificial attrition varied with the diet. Thus, the teeth of an animal on a diet containing an adequate quantity of vitamin D responded by producing abundant well formed secondary dentine. On the other hand, a diet containing little vitamin D, particularly in relation to the amount of cereal, resulted with the same stimulus in the production of either no secondary dentine, or of dentine of a defective quality containing interglobular spaces. It appeared that the resistance and type of reaction of the teeth to a harmful stimulus varied according to their state of nutrition at the time of the stimulus, whatever their original structure may have been. Since the same histological changes are found in human teeth resulting from attrition and caries, it seems fair to infer that their resistance to attack also varies with differences in nutrition. Of 1000 deciduous teeth examined, in only comparatively few cases was caries present in a normally calcified tooth and in those the secondary dentine was nearly always absent or defective in structure, suggesting that the diet had been changed from one with strong calcifying properties during the developmental period, to one lacking such properties after eruption. On the other hand, when a hypoplastic tooth was free from caries, any secondary dentine formed in response to attrition was usually abundant and well calcified, indicating the reverse conditions of nutrition.

EVIDENCE THAT THE STRUCTURAL VARIATIONS IN TEETH WHICH ARE CONTROLLED BY DIET ARE OF SIGNIFICANCE IN RELATION TO CARIES. There can be little doubt that the structure of human teeth and their susceptibility to caries are intimately related. In the examination of over 1000 deciduous and many permanent teeth, M. Mellanby (78) found that of those which were normal or nearly normal in structure, only 27 per cent had carious cavities as compared with 85 per cent in the hypoplastic teeth. Again the incisors were not only better calcified than the molars, but were also much freer from caries. In fact, nearly 90 per cent of the teeth examined might be considered to support the general hypothesis that structure and caries are intimately related. The character of the secondary dentine in the exceptions suggests that the majority of these may be due to altered resistance of the living teeth after eruption.

Although this review deals with the effect of diet on the structure of teeth, yet a few words must be said on the related problems of the effect of diet on the development and spread of caries. Experiments on rats, guinea pigs and monkeys, carried out by several workers, have resulted in the experimental production of caries-like lesions in some cases.

In 1922 McCollum, Simmonds, Kinney and Grieves (44) reported an investigation on lesions found in the teeth of rats fed on deficient diets varying primarily in the amount of vitamin A, calcium, phosphorus and protein. They found "caries-like" lesions in over 5 per cent of rats fed on a low calcium and fat-soluble vitamin diet, under 2 per cent in those fed on diets abundant in fat-soluble A, whether the calcium was high or low, but none in their stock animals fed on a mixed diet. Grieves (45) later described these results in more detail. He made sections of some of the teeth showing caries-like lesions and states that these show areas of enamel decalcification and enlargement of the dentinal tubes. Toverud (46) found some macroscopic lesions resembling caries in the teeth of rats fed on diets low in calcium and fat-soluble vitamin A. Howe (79), (80), (81) has made extensive investigations chiefly on guinea pigs and monkeys and finds changes resembling caries when the diets are deficient in vitamin C. In a recent report (82) he states that "the most rapid and pronounced caries in monkeys has occurred when the diet has been largely composed of gelatin and cereals; that a deficiency of the antiscorbutic factor has played a part in the production of caries; that excess of sugars and starches has not resulted in caries in nine months; that monkeys fed on milk, vegetables and fruit diet have excellent teeth." Marshall says that in experiments on dogs he did not find any evidence of the development of caries (61) but he reports (83) the presence of carious lesions in rats fed on diets deficient in vitamin A. These lesions only developed in the older animals and never in the young. He was unable to arrest the progress of caries when an animal on a previously vitamin A deficient diet, was later given one containing vitamin A.

If the claims of these investigators can be substantiated they go a long way to prove the relationship between certain specific dietetic factors and caries. The writer's attempts to produce caries in the teeth of dogs, rabbits and rats have only met with partial success, and have not allowed the subject to be systematically studied by this type of experimental procedure, so that she has herself but little direct evidence of the relation of structure to caries.

Indirect evidence has, however, been obtained by observations on the

rate of spread of caries in children when fed on different diets (M. Mellanby and C. Lee Pattison, 84, 85). Three groups of 35 children were fed *a*, on diets which, on the basis of the previous work should ensure good calcification, i.e., the diets included a high milk intake, eggs and cod liver oil; *b*, on diets with definitely lower calcifying properties, including less milk, oatmeal and olive oil substituted for cod liver oil, and *c*, the ordinary institutional diet with calcifying properties intermediate between *a* and *b*. The teeth were charted for the amount and character of dental caries at the beginning of the special dietary and again after an average period of about eight months. The following results (Table 6) were obtained.

It seems evident from this investigation that the incidence and spread of caries in the teeth of children can be directly influenced by varying those substances in the diet which have been shown above to influence

TABLE 6

Effect of diet on caries in children

DIET	NUMBER OF CHILDREN IN GROUP	MAIN DIETETIC DIFFERENCES	AVERAGE NUMBER OF TEETH PER CHILD IN WHICH CARIES HAS SPREAD
A	32	Abundant calcifying vitamin D and calcium; no oatmeal	1.7
B	34	Less vitamin and calcium: some oatmeal	5.6
C	37	Intermediate between A and B	3.0

dental structure and in addition to alter the resistance of the teeth to harmful stimuli. Further work of this type on a large scale is now in progress in England and it ought to be possible in the near future to narrow down the conditions so as to lead to a more accurate corroboration of the earlier investigations.

One final piece of evidence may be adduced to support the views advanced. When teeth have developed caries and the infective process is subsequently arrested, it is usual to find a large amount of well calcified secondary dentine (77) such as has been shown above to be produced in animals during irritation of the enamel when the diet has intense calcifying properties.

ARE THE EXPERIMENTAL RESULTS IN KEEPING WITH HUMAN EXPERIENCE? The time comes when all physiological evidence must be referred to, or compared with human experience, and this is especially so

when it touches on human disease. As has already been mentioned, some of the results of the experiments on puppies have been extended to children and have been found to hold good. Can the same be said in attempting to explain the condition of the teeth of different nations and races of people on the basis of these experiments? Do the facts explain, for instance, why the teeth of the English and American people are so bad and the teeth of natives living in tropical lands, or those of the Esquimo in his natural habitat are comparatively good? Can they account for the statement that the teeth of paleolithic man were good and that with the advent of civilisation they have degenerated; also for the fact that, in England, people brought up under better social conditions tend to have better formed teeth than the poor? Many more investigations must be carried out before these questions can be fully answered. It is certainly necessary to have more evidence as to the structure of the teeth of different races, but nevertheless it is probable that recently gained knowledge can be used to explain some of the facts.

It has been pointed out that the most important specific influences affecting the structure of the teeth during development are *a*, the intake of vitamin D or the exposure of the skin to sunlight or other sources of ultra-violet radiation, and *b*, the amount and type of cereal eaten. The first of these promotes and the second hinders calcification of teeth.

Now in England the climate is relatively sunless; moreover, the atmosphere is such as to preclude the possibility of ultra-violet radiations piercing to the earth's surface to any great extent. Again, the climate is sufficiently cold to make it necessary to cover the skin with clothing. Thus there is but little chance of ultra-violet radiations having much influence on the calcification of the teeth. What about the intake of vitamin D in the food? For this we rely largely on milk, butter, whole-milk cheese and eggs. The consumption of milk is notoriously low, statistical evidence recording that the average daily consumption in England is about 150 cc. Butter and eggs are not consumed in any large quantity by the majority of people. Another reason, also consistent with the experimental results, which helps to explain the defective structure of English teeth, is the large part played in the dietary by cereals and cereal products. Cereals are cheap, easily stored and transported, and have many qualities which make them of fundamental importance in the diet of an urban population dependent largely on imported food. They have the great defect of being not only deficient

in fat-soluble vitamins but of actively interfering with calcification processes. They create, in fact, a greater demand on "protective" foods containing vitamin D, and in England, at least, this demand is not satisfied.

It may be asked why the Indians and others living in tropical countries where the diet is mainly composed of cereals such as rice and millet are said to have comparatively good teeth. The answer to this is supplied partly by the experiments showing the effect of ultra-violet radiations on tooth structure. Where there is abundant sunshine it is possible that the anticalcifying effect of cereals may be completely antagonized. When the native of tropical lands comes to live in temperate zones and shares the life of civilized man, his teeth suffer the general decadent changes of his newly adopted community.

The Esquimo falls into a different category. His natural diet is composed largely of fish, blubber and meat and includes little or no cereal. It is true that he gets less sunshine than the people of the temperate zones, but the greater intake of vitamin D in the fish, fat and blubber, and the smaller intake of cereals accounts for good tooth calcification. Here again when he leaves his natural habitat and lives under the conditions associated with trading stations or civilized communities, his teeth are said to degenerate, as might be expected on the basis of the above mentioned investigations.

The degeneration of the teeth which has accompanied the advance of civilisation can probably be explained on the same basis. Since paleolithic times one of the greatest changes affecting human life has been the introduction of cereals into the diet of man and the gradual increase of their consumption, until to-day they are said to form 60 per cent of the diet of the average person. With the increase in the cereal consumed there has been a corresponding diminution in the eating of animal products, including meat, fish, milk and eggs. Wherever the consumption of these vitamin-containing foods remains high, as was the case until recently in the Hebrides, the teeth are conspicuously well formed. This fact was commented upon in the Carnegie Report of the Physical Welfare of Mothers and Children of Scotland. It is true that a hundred years ago the diet of the average Scot included much oatmeal, but it is equally true that he consumed with it, among other vitamin-D-containing foods, large quantities of milk. Abundant milk can antagonize any detrimental effect oatmeal has on the structure of the teeth.

Another change in mode of living which might be expected to have detrimental effects on the teeth, is the decrease in England and many

other countries of breast feeding. The relative infrequence and shortness in duration of breast feeding in modern times, and the practice of feeding infants on cereal concoctions might be expected to lead to defective teeth.

Evidence has also been given to show that in England the teeth of the well-to-do are, on the whole, rather better formed than those of the poor, although both are defective. Undoubtedly the explanation lies in the fact that poverty makes it necessary that a larger proportion of the diet should consist of cereals. The differences might be even greater were it not for the fact that it is the rule for the children of poorer people to be breast fed, whereas among the well-to-do this practice is not nearly as common. It is said that in certain districts such as in the Lancashire industrial towns, where woman-labour is extensively employed and breast feeding among the poor is not so common, the teeth are deplorable.

Although much work remains to be done, it will probably be agreed that the results, obtained from the animal experiments made with the object of determining the conditions which produce normal and hypoplastic teeth, are in keeping with human experience.

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